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METABOLITES OF *ARMILLARIA MELLEA* AND SYNTHETIC STUDIES ON A POTENTIAL IRON-BINDING FUNGAL METABOLITE AND ANALOGUES

by

JOHN BEVERLEY MACAULAY

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF CHEMISTRY

EDMONTON, ALBERTA

FALL 1986

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Supervisor

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External Examiner

Date..... May 29, 1986 .....

To Rosa and my parents

ABSTRACT

Studies on the metabolites of forest disease fungi are presented in this thesis.

The fungus Armillaria mellea (Vahl ex. Fr.) Kummer is responsible for severe losses in timber and fruit production. The first part of this thesis describes the isolation and structural elucidation of some of the metabolites produced when certain strains of this fungus are grown in liquid culture. The metabolites have been identified as diterpenoid acids possessing the abietane (30) and pimarane (33) skeletons. These compounds, known collectively as resin acids, have not been reported previously from a fungal source. The resin acids obtained include dehydroabietic acid (31), pimaric acid (34), isopimaric acid (36), sandaracopimaric acid (38), levopimaric acid endo-peroxide (40), 7-oxodehydroabietic acid (43), and 7-oxo-15hydroxydehydroabietic acid (45). On one occasion three orange pigments, austocystin F (46), averufin (48), and averufanin (50), all previously known fungal metabolites, were isolated.

Blue stain disease of conifers currently causes the death of about 40 million trees annually in Western Canada. The complex of fungi which cause the disease belong to the genus Ceratocystis. The blue staining of the sapwood of infected trees appears to be due to the iron chelates of metabolites produced by the fungi involved. The second part of this thesis describes the synthesis of the catechol-type

analogues (12-15) of the resorcinol-type Ceratocytis metabolites 6-9. Each of the enantiomers of 12 was prepared separately in order to prove the absolute configuration of the natural product 7-hydroxymellein (18). The catechol moiety in structures 12-15 and 18 make them potential iron-binding compounds.

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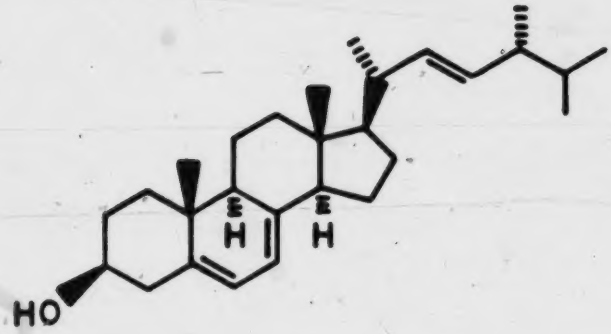
1. INTRODUCTION

The fungus Armillaria mellea (Vahl ex. Fr.) Kummer, also known as shoe string root rot (the fruiting body is the "honey mushroom"), is a vigorous pathogen of many commercially important types of trees[1]. Larch, cedar, willow, walnut, and apple trees are all very susceptible to attack by this fungus. Fir, oak, and birch, although frequently attacked, show fewer adverse effects. The fungus invades the healthy tree via the roots. Sheets of white mycelium develop just beneath the outer bark as the fungus spreads. The mycelium may completely girdle small trees and shrubs. This girdling, combined with extensive damage to the root system, deprives the leaves of water, and the tree wilts and dies[1,2].

Because of our interest in forest disease fungi, we undertook to investigate metabolites of A. mellea as part of a collaborative research project with scientists at the Northern Forest Research Centre (Edmonton, Alberta). Our interest was further stimulated by the fact that several metabolites of A. mellea, which showed antifungal and antibiotic activity, were reported by Oduro[3,4].

Previous chemical studies on A. mellea were not

extensive. Ergosterol (1) and unsaturated analogues of



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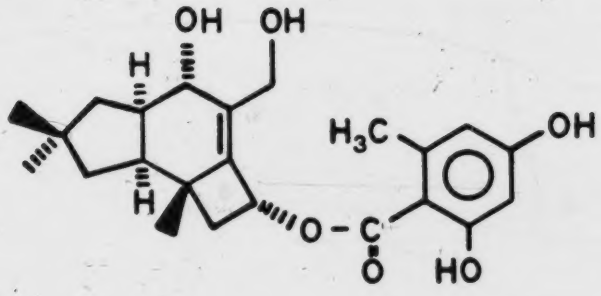
ergosterol were detected in the acetone extracts of the fruiting bodies[5]. Water-soluble polysaccharides were



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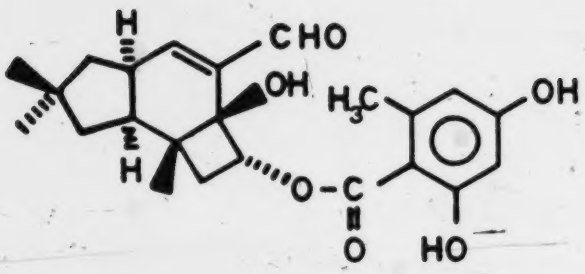
obtained from the fruiting bodies[6] and the mycelium[7].



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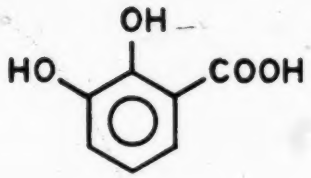
However, during the tenure of our study, other examinations of the metabolites of A. mellea were published. These reported orsellinate sesquiterpenoids of the protoilludane[8] skeleton, including armillol (2)[9], a neoilludol derivative[10], and melleolide (3)[11]. Armillol, which shows antibiotic activity, was isolated from mycelial extracts, while extraction of liquid cultures afforded melleolide, which exhibits antibacterial and antifungal activity.

The first part of this thesis describes the isolation and structural elucidation of some metabolites afforded by three strains (NFRC-621, 624, and 631) of A. mellea,

indigenous to western Canada, when they are grown in liquid culture. It is anticipated that the results obtained may contribute to the advancement of basic knowledge concerning this forest pathogen and perhaps aid in the development of a long-term solution to this problem.

A complex of fungi[12] belonging to the genus Ceratocystis causes a disease of conifers known as blue stain[13], a designation arising from the fact that the sapwood of the afflicted trees is stained a pronounced blue colour. The disease is currently responsible for the deaths of about 40 million trees annually in western Canada[14]. As the blue stain fungi spread throughout and gradually encircle the sapwood of the tree, the structure above the infected area becomes deprived of water. As a result, the tree rapidly wilts and dies[15,16]. Recently it has been reported[16,17] that as the blue stain develops in the sapwood, the transpiration system of the infected tree becomes restricted to the inner portions of sapwood and eventually transpiration fails completely. Since the onset of water deprivation may coincide with the appearance of the blue colour, the fungal metabolites which may be responsible for the staining effect are of particular interest. A study conducted in this laboratory on the various fungi thought to be involved in the disease has resulted in the isolation of 2,3-dihydroxybenzoic acid (4)[18] and the novel natural product ceratenolone (5)[19] from C. huntii and C. minor, respectively. 2,3-Dihydroxybenzoic acid (4) is a known

iron-chelating agent[20] and it gives a bright blue colour in the presence of ferric chloride. Ceratenolone (5) also possesses the structural characteristics required for iron complexation and has been shown to form an iron chelate very readily. Thus it appears likely that the blue staining of



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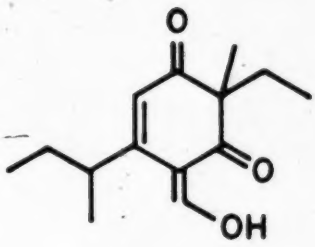

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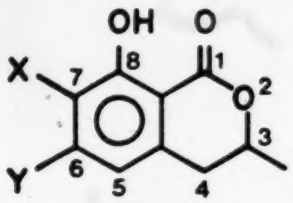
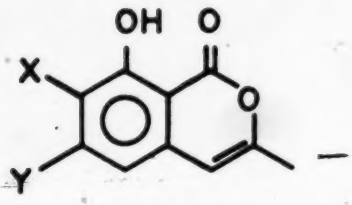
the wood is attributable to the iron chelates of these two compounds and perhaps other, not yet isolated metabolites of the blue stain fungi.

Microbial metabolites which function as iron transport agents are called siderophores[21]. Almost invariably these compounds incorporate ortho-acyl-substituted catechols (such as 4) or hydroxamic acids as the structural features which serve as ligands to coordinate the metal ion. Ceratocystis species produce a series of structurally similar isocoumarin

metabolites, including 6 (C. minor[22]), 7 (C. minor[22,23], C. fimbriata[24], C. ips[18]), 8 (C. minor[22], C. ulmi[25]), 9 (C. minor[23], C. ips[18]), 10 (C. minor[22], C. ulmi[25]), and 11 (C. ulmi[25]).



6 X = H Y = OH
12 X = OH Y = H



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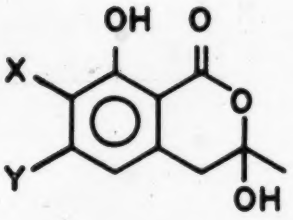
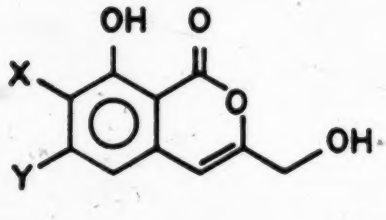
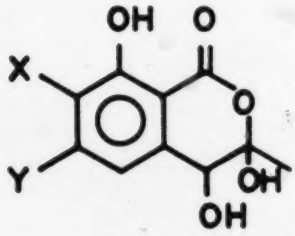


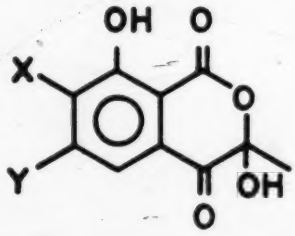
8 X = H Y = OH
14 X = OH Y = H



9 X = H Y = OH
15 X = OH Y = H



10 X = H Y = OH
16 X = OH Y = H

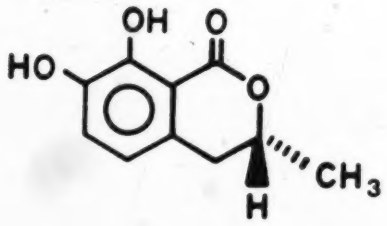


11 X = H Y = OH
17 X = OH Y = H

These compounds would exhibit the ortho-acyl-substituted catechol moiety, thus endowing them with

iron-binding (and presumably staining) capabilities, if not for the 6,8 (resorcinol) instead of 7,8 (catechol) arrangement of the two phenols. In light of the fact that production of iron-chelating compounds by blue stain Ceratocystis species has been established, it occurred to us that the 7,8-diphenolic analogues of 6-11 also might be metabolites. We decided to synthesise these analogues, compounds 12-17, so that their relative iron-binding capacities could be measured and their possible role in tree mortality and symptom expression studied in in vivo bioassays. No synthetic efforts towards compounds 12-17 have appeared. Also, having authentic samples of 12-17 in hand would very much simplify the detection of these compounds in crude extracts of the blue stain fungi.

The proposal to prepare 12-17 found additional justification in that the levorotary enantiomer of 12,



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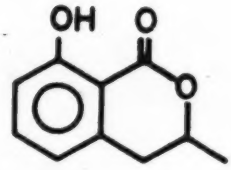

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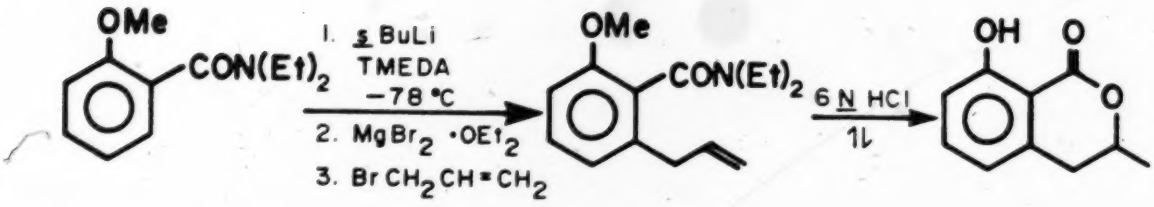
(R)-(-)-7-hydroxymellein (18), already has been isolated as a natural product[26] (although not from a Ceratocystis species). The R configuration has been assigned to the naturally occurring compound, however we felt it desirable

to determine unequivocally the absolute configuration by synthesis of chiral 18. The fact that no synthesis of the natural product 18 has been reported provided added incentive for its preparation.

Scheme I **Snieckus' synthesis of mellein (19)**

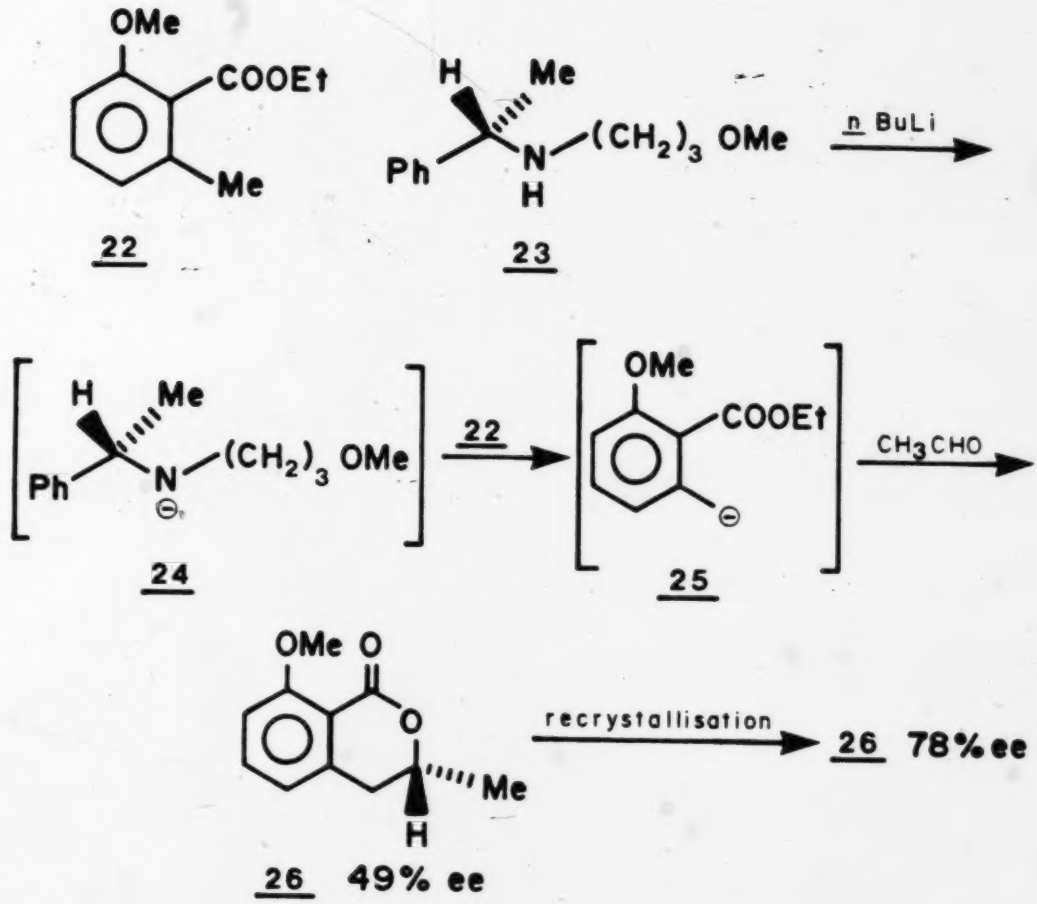


20 21 19

In planning a synthetic approach to 12-18, we examined previous preparations[26-31] of mellein (19), the 7-dehydroxy analogue of 18. We required a method which would permit creation of the proper level of oxidation in the lactone ring of each target compound. The methodology of Snieckus (Scheme 1)[31], who prepared mellein via addition of a three-carbon unit to an appropriately substituted aromatic nucleus 20, was deemed most relevant to the synthetic problem. Clearly, 12 could be synthesised in analogous fashion by employing an aromatic ring having an additional methoxy substituent. Manipulation of functionality in a three-carbon side chain (21) should allow achievement of the requisite oxidation level for each of 13-17. Since keto-lactol 11 has been converted into hydroxy-lactol 10[32,33], synthesis of 17 would also provide access to 16 by

an analogous transformation.

Scheme 2 Regan's asymmetric synthesis of (26)

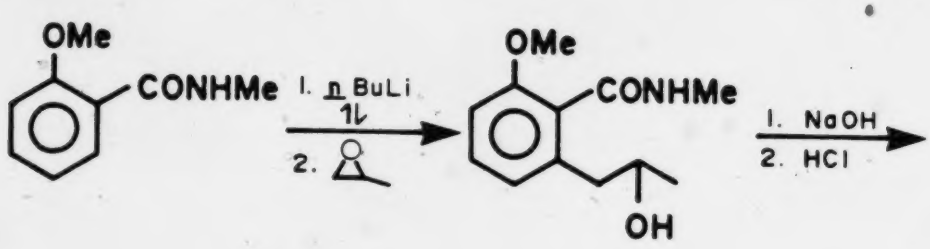


In order to synthesise 18, the introduction of an element of chirality would be necessary. Regan (Scheme 2)[34] employed an ortho-toluate carbanion (25), generated by a chiral base (24), in the asymmetric synthesis of mellein methyl ether (26). However, this methodology requires the use of a chiral shift reagent to determine (by ^{1}H nmr) which

enantiomer of the product is formed, and the degree of enantiomeric purity is not exceptionally high.

In preparing mellein (19), Narasimhan (Scheme 3)[27] appended a three-carbon unit, in the form of propylene oxide, to an aromatic nucleus (27). Since propylene oxide is commercially available in chiral form, we proposed to use it in Snieckus' sequence (vide supra) to introduce the chiral centre needed for the synthesis of 18. The second part of this thesis details these synthetic studies.

Scheme 3 **Narasimhan's synthesis of mellein (19)**



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29 19

2. METABOLITES OF *ARMILLARIA MELLEA*

2.1 DISCUSSION AND RESULTS

Liquid cultures of Armillaria mellea, strains NFRC-621, 624, and 631, were grown on either 10% (v/v) clarified V-8 juice containing 1% (w/v) glucose or on potato dextrose broth. After a growth period of between 34 and 92 days, the mycelial mat was removed by filtration. The culture broth was then extracted with chloroform (the same solvent used by Oduro[3]). The mycelium was separately subjected to Soxhlet extraction with chloroform or methanol.

The crude broth extract of strain NFRC-621 was examined by thin layer chromatography (tlc).' Oduro's solvent system (hexane-ethyl acetate-methanol, 80:20:1) was employed, but the tlc obtained differed from that reported[3]. The tlc appeared to reveal one major (least polar) component and several minor components. All of these absorbed ultraviolet (uv) light (254 nm). The same components were indicated by tlc of the crude chloroform extracts of strains NFRC-624 and 631.

Separation of the crude metabolites into neutral and acidic components was attempted by extraction with 10% (w/v) sodium bicarbonate. However, the neutral and acidic fractions isolated were very similar by tlc.

A ferric chloride test for phenols in the crude extract was negative.

'All references to tlc behaviour of Armillaria mellea metabolites shall imply silica gel as the adsorbent and 10% or 30% (v/v) sulphuric acid solution followed by charring as the visualisation method.

Sephadex chromatography did not separate the components of the crude extract.

Flash chromatography[35] provided a satisfactory method of separating the major, non-polar material from the mixture of crude metabolites. High resolution mass spectroscopy (hrms) indicated that this material was composed of a mixture of compounds possessing molecular formula $C_{20}H_{30}O_2$ (m/z 302, 94% intensity) and $C_{20}H_{28}O_2$ (m/z 300, 57%). Molecular weights of 302 and 300 were confirmed by chemical ionisation mass spectroscopy (cims). Thus peaks were observed at m/z 320 (302+18), 318 (300+18), and 303 (302+1). The infrared (ir) spectrum of the mixture showed broad OH absorption (3300-2400 cm^{-1}) and strong carbonyl absorption (1692 cm^{-1}) indicative of carboxylic acids. The ^1H nuclear magnetic resonance (nmr) spectrum contained five peaks between δ0.85 and δ1.25, suggesting the presence of methyl groups. Signals for both olefinic and aromatic protons were also observed. Esterification with diazomethane converted the components of the mixture to the corresponding methyl esters (the ir spectrum revealed no OH absorption, while the carbonyl band was shifted to 1720 cm^{-1}). The cims gave peaks at m/z 334 (316+18) and 317 (316+1) corresponding to methylation of the $C_{20}H_{30}O_2$ compound(s) and m/z 332 (314+18) resulting from formation of the methyl ester of the $C_{20}H_{28}O_2$ component(s). The ^1H nmr spectrum of the mixture of esters was almost identical with that of the acids except for the appearance of methyl singlets at δ3.63, 3.66, and 3.67.

Mixtures of carboxylic acids are often transformed into the corresponding methyl esters in order to facilitate separation by conventional silica gel or alumina chromatography. However, tlc analysis of the mixture of esters obtained indicated that they were no more readily separable than the parent acids.

Hydrogenation (H_2, 10% Pd/C) of the mixture of acids resulted in the uptake of four atoms of hydrogen by the $C_{20}H_{30}O_2$ component(s) but left the $C_{20}H_{28}O_2$ compound(s) unchanged. This result was indicated by cims, which gave peaks of m/z 324 (306+18) and 318 (300+18).

Separation of the mixture of acids was attempted by fractional crystallisation from 95% ethanol. After three recrystallisations fine, white crystals were obtained. However, hrms showed (m/z 302, 100% and 300, 31%) that the components of the mixture were co-crytallising.

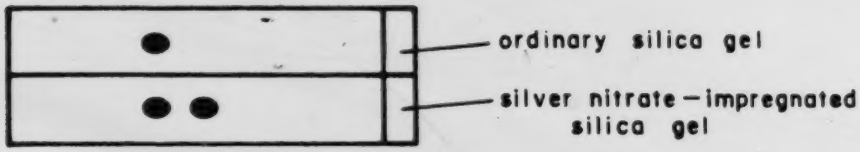
Extensive efforts were made to separate the acids by high pressure liquid chromatography (hplc) but these were also unsuccessful.

Silica gel impregnated with silver nitrate has been used in the chromatographic separation of olefinic compounds[36]. It was discovered that the mixture of acids, which appears as a single spot by ordinary silica gel tlc, could be resolved into two spots by adsorption of silver nitrate onto the silica gel (Scheme 4). A partial

purification of these two spots was achieved by flash

Scheme 4 **Separation of acids into two spots (tlc)**

Skellysolve B – ethyl acetate – acetic acid, 75:25:1, 1x

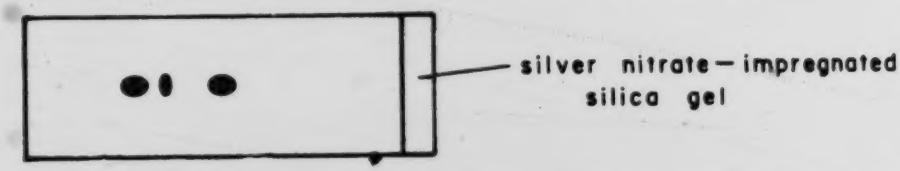


chromatography using silica gel impregnated with 5% (w/w) silver nitrate. From the ^1H nmr spectrum of the upper (less polar) material, it was evident that this spot was comprised of the aromatic constituent(s) of the mixture. Likewise a sample of the more polar spot displayed olefinic (but no aromatic) protons by ^1H nmr. The olefinic acids were confirmed as the $C_{20}H_{30}O_2$ components of the mixture by ms (m/z 302, 100%).

It was discovered that the mixture of acids appeared as three distinct spots by tlc when the following solvent system was used: chloroform-ethyl acetate-acetic acid, 97:2:1, 3x (Scheme 5). By employing this solvent system and

Scheme 5 **Separation of acids into three spots (tlc)**

chloroform – ethyl acetate – acetic acid, 97:2:1, 3x



ms, it was shown that the same mixture of carboxylic acids was present in the crude broth extracts of strains NFRC-624

and 631, thus indicating a chemical similarity between the three strains. Strain NFRC-631 also gave rise to a metabolite not observed by tlc in the other two strains (vide infra).

A gravity column containing silica gel impregnated with silver nitrate (17% w/w) was used successfully to separate the three components representing the mixture of acids. The least polar material,[2] previously shown to be aromatic, proved to be a single compound. It was demonstrated to be the $C_{20}H_{28}O_2$ component of the mixture by ms. Inspection of the ^1H nmr spectrum (Figure 1) reveals that the $C_{20}H_{28}O_2$ component contains a 1,2,4-trisubstituted benzenoid nucleus and that one of the substituents is an isopropyl group. Thus the three aromatic protons (δ6.87, 6.98, 7.14) possess ortho (J=8 Hz), ortho and meta (J=8, 1.75 Hz), and meta (J= 1.75 Hz) couplings respectively. Three benzylic protons are evident, two as a multiplet centred at δ2.90, and an isopropyl methine appearing at δ2.82 (septet, J=7 Hz). The ir spectrum shows absorptions for a carboxylic acid at 3200-2400 cm^{-1}(OH) and 1692 cm^{-1}(C=O). The fragmentation pattern of the mass spectrum is indicative of an abietane skeleton 30[8], (as judged by comparison with mass spectra of other compounds which possess the abietane framework). Comparison (tlc, ^1H nmr, and ir) with an authentic sample showed that this component is dehydroabietic acid (31). The optical rotation

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[2]However, on ordinary silica gel 31 is the most polar of the mixture of acids; see Experimental section.

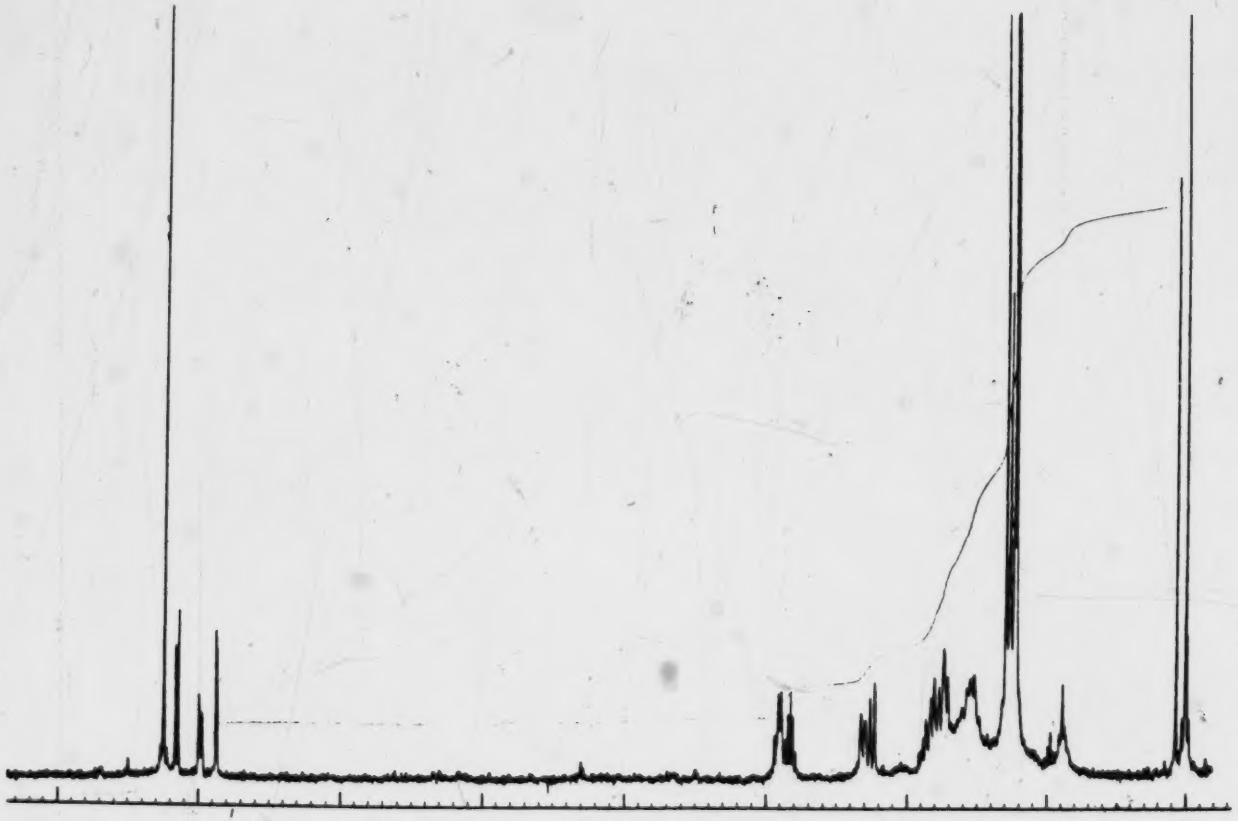
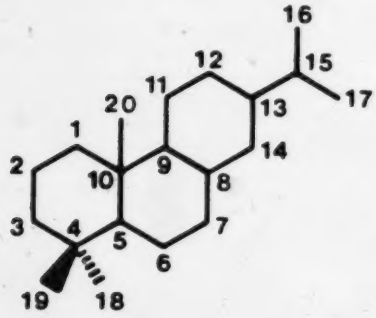


Figure 1. 400 MHz ^{1}H nmr spectrum ($CDCl_3$) of dehydroabietic acid (31)

($[\alpha]_D$ +54°) confirmed the absolute configuration[37]. In order to demonstrate the presence of dehydroabietic acid (31) in the crude extract of strain NFRC-621, the ^1H nmr spectrum of the crude extract was obtained, then 31 was added to the sample and the spectrum was rerun. No new signals appeared, while those corresponding to 31 increased in intensity.



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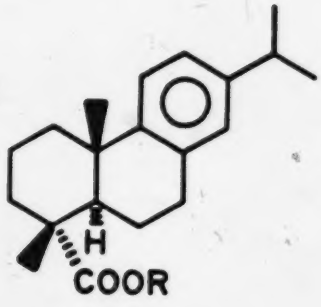


31 R = H

32 R = Me

The spots having R_f 0.65 and 0.52 (Scheme 5) also eluted separately from the gravity column of silver nitrate-impregnated silica gel. These isomeric compounds comprised the olefinic ($C_{20}H_{30}O_2$) portion of the original mixture of acids (vide supra). This was confirmed by hrms (m/z 302, 49% and 302, 100% respectively). From the ^1H nmr spectra (Figures 2 and 3) it was apparent that the component of R_f 0.65 was a single compound (34), while the more polar material was an inseparable mixture of two compounds (36 and (38). The ^1H nmr spectra of all three components show an AMN splitting pattern in the region δ4.8-5.9 characteristic

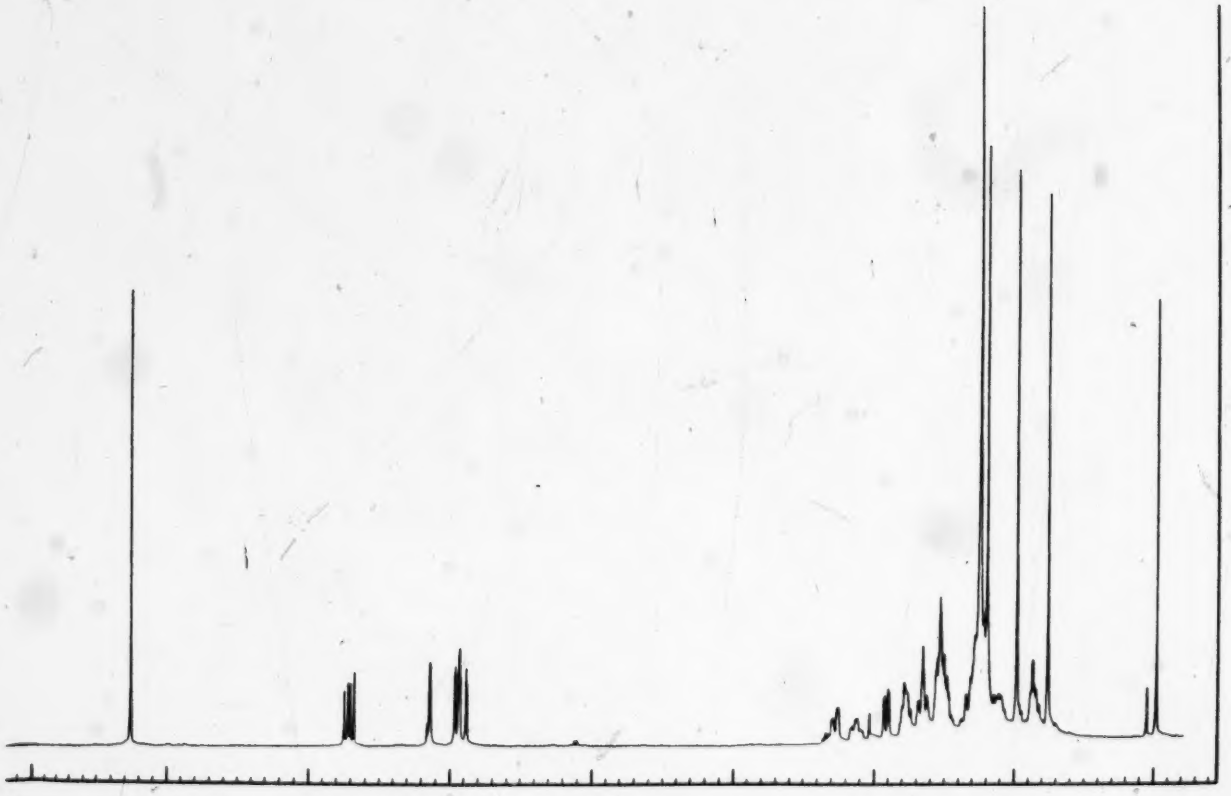


Figure 2. 400 MHz ^{1}H nmr spectrum ($CDCl_3$) of pimaric acid (34)

of a vinyl group. This suggested that compounds 34, 36, and 38 possess the pimarane skeleton 33[8], which is closely related biosynthetically[38] to the abietane skeleton 30. The presence of an additional olefinic proton (see Table 1) indicated that each of the three components possesses a trisubstituted (nuclear) carbon-carbon double bond.

Table 1 ^{1}H nmr data of olefinic protons, 34, 36, 38

Compound	Proton	δ	multiplicity	J (Hz)
34	H_A	5.72	dd	17.5, 10.5
	H_B	4.96	dd	10.5, 2
	H_C	4.91	dd	17.5, 2
	$H_{nuclear}$	5.15	bs	—
36	H_A	5.81	dd	17.5, 10.5
	H_B	4.87	dd	10.5, 1.5
	H_C	4.93	dd	17.5, 1.5
	$H_{nuclear}$	5.33	bd	5
38	H_A	5.78	dd	17.5, 10.5
	H_B	4.89	dd	10.5, 1.5
	H_C	4.91	dd	17.5, 1.5
	$H_{nuclear}$	5.22	bs	—

The equatorial disposition of the carboxyl group in the three compounds was confirmed [39] by the C-O stretch (1244 cm^{-1}) in the ir spectra of the corresponding methyl esters (formed by treatment of the acids with diazomethane). It has been observed [40] that the ir frequency of the out-of-plane C-H bend of the vinyl group in pimarane-type

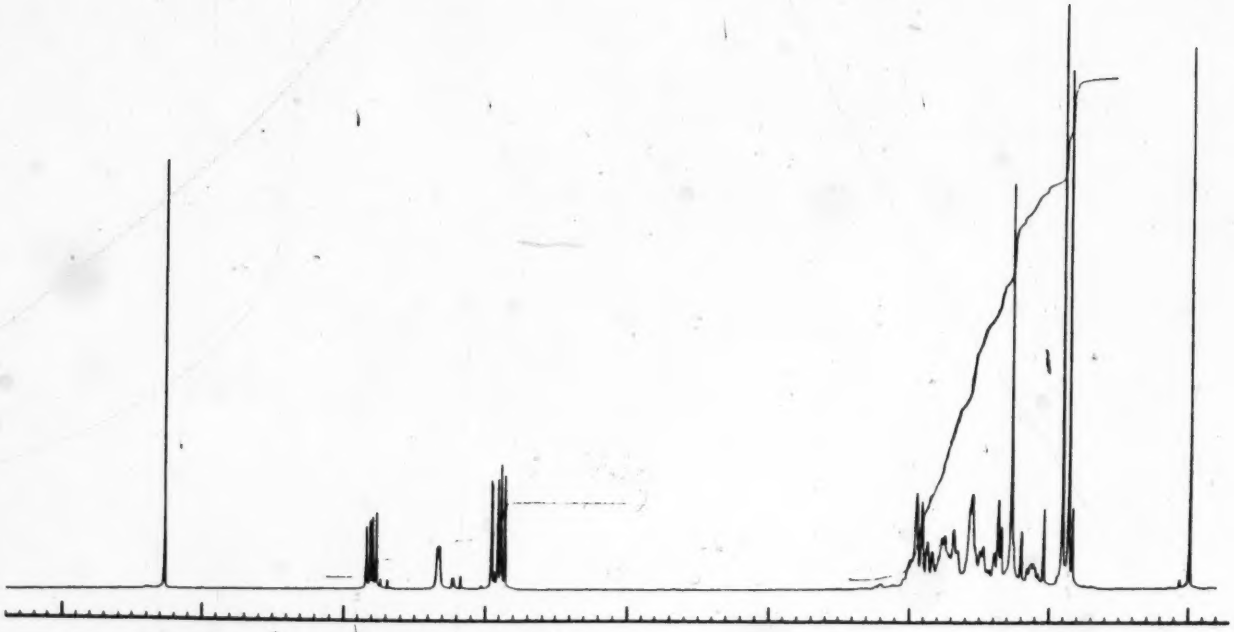
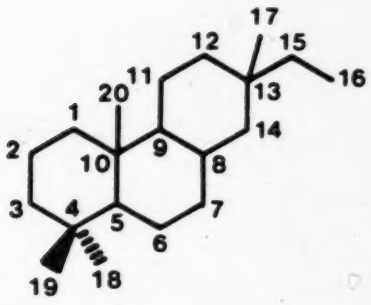
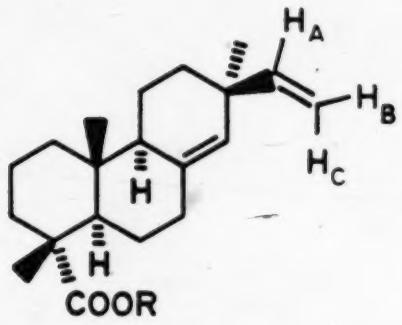


Figure 3. 400 MHz ^{1}H nmr spectrum ($CDCl_3$) of isopimaric acid (36) and sandaracopimaric acid (38)

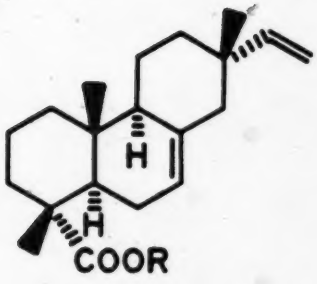
structures is particularly diagnostic with respect to the relative stereochemistry of the vinyl and methyl groups



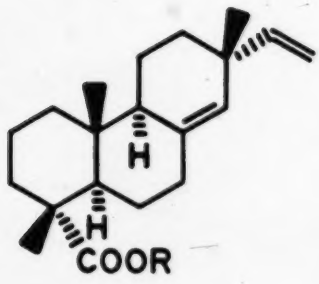
33



34 R = H
35 R = Me



36 R = H
37 R = Me



38 R = H
39 R = Me

attached to C-13. Thus a 914 cm^{-1} absorption in the ir spectrum of the methyl ester 35 indicates a β-vinyl substituent group while the mixture of the methyl esters 37 and 39 shows absorption at 909 cm^{-1}, suggesting an α-vinyl substituent. These data led to the assignment of the three isomers as pimaric (34), isopimaric (36), and

sandaracopimaric (38) acids respectively. Identification was confirmed by comparison (tlc, ^{1}H nmr) with authentic samples. The ^{1}H nmr signals of the major component in the mixture of 36 and 38 are superimposable with those of isopimaric acid (36), while the signals corresponding to the minor compound are in excellent agreement with those of sandaracopimaric acid (38).

Separation of the methyl esters of resin acids over neutral alumina impregnated with silver nitrate has been reported[41]. Thus the mixture 31, 34, 36, and 38 was esterified with diazomethane. Chromatogaphy using a gravity column employing neutral alumina containing 17% (w/w) adsorbed silver nitrate was performed on the esters. The esters 32 and 35 eluted in mixed fractions, however the latter fractions afforded a mixture (one spot by tlc using silver nitrate impregnated silica gel) of the esters 37 and 39.

Additional metabolites were isolated from the more polar fractions of the crude broth extracts. One of these, compound 40, appeared as a major component of the broth extract from strain NFRC-631 but was not present in the broth extracts of the other two strains. Compound 40 contains a carboxy function which can be methylated (diazomethane) to give 41, resulting in a shift of the carbonyl frequency in the ir spectrum from 1692 cm^{-1} to 1720 cm^{-1}. In the ^{1}H nmr spectrum of 40 (Figure 4), a septet at δ2.50 (J=7 Hz) again suggests an isopropyl group and thus

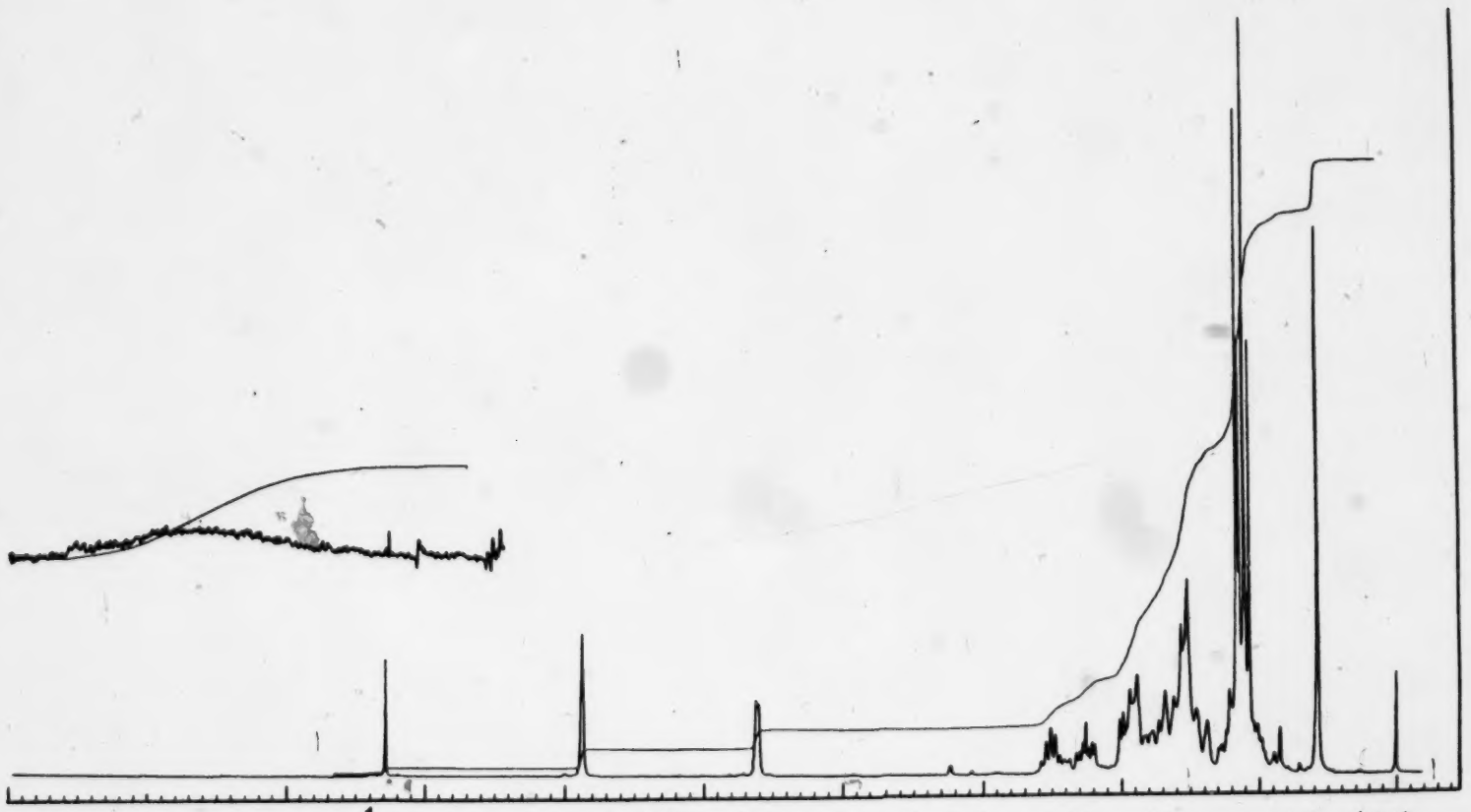
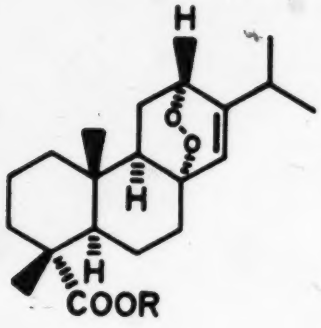
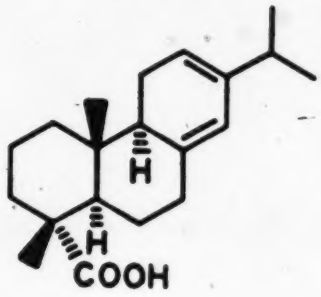


Figure 4. 200 MHz ^{1}H nmr spectrum ($CDCl_3$) of levopimaric acid <u>endo</u>-peroxide (<u>40</u>)

the abietane skeleton. It is noteworthy that the methyl groups of the isopropyl unit in 40 give rise to two well separated doublets, δ1.08 and 1.12 (J=7 Hz), indicating that the environments of the two methyls differ more than in 31, where the isopropyl methyl doublets overlap (δ1.22, J=7 Hz).



40 R = H
41 R = Me

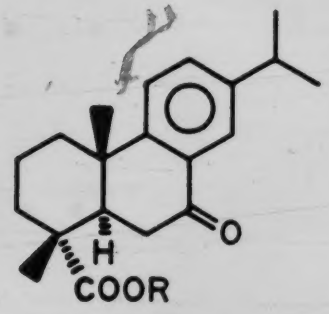


42

Compound 40 has one olefinic proton appearing as a singlet at δ5.87. Assuming the abietane skeleton and since the isopropyl methine is allylic (δ2.50), the singlet nature of the olefinic proton indicates a $\Delta^{13,14}$ double bond with C-8 fully substituted. The appearance of signals at δ76.9 (s) and δ74.6 (d) in the ^{13}C nmr spectrum indicates the presence of two oxygenated sp^3 carbons. This, coupled with the fact that the base peak in the mass spectrum (m/z 302) corresponds to the loss of a molecule of oxygen, suggested that 40 might be the endo-peroxide of levopimaric acid (42). Comparison of the methyl ester 41 with an authentic sample confirmed the identity. Although the endo-peroxide is not an auto-oxidation product [42] of levopimaric acid (42), the

presence of light and a photosensitising substance in the crude extract [43] may bring about the incorporation of molecular oxygen into the homoannular diene of 42.

Following the isolation of dehydroabietic acid (31), it was noted that the tlc behaviour of one of its decomposition products corresponded to that of a component present in the crude broth extract. This compound (43) was obtained in the flash chromatography fractions eluting after the acids 31, 34, 36, and 38. Its isolation from the complex mixture of crude metabolites was somewhat facilitated by the fact that this substance stands out as a bright white spot on tlc when



43 R = H
44 R = Me

viewed under uv light (350 nm) after charring. Final purification of 43 was carried out by preparative tlc. The ^{1}H nmr spectrum (Figure 5) reveals three aromatic protons with a coupling pattern similar to that of 31, although at somewhat lower field. A benzylic isopropyl methine proton appears at δ2.92 (septet, J=7 Hz), but the C-7 benzylic protons of 31 are absent. A ketonic carbon is evident in

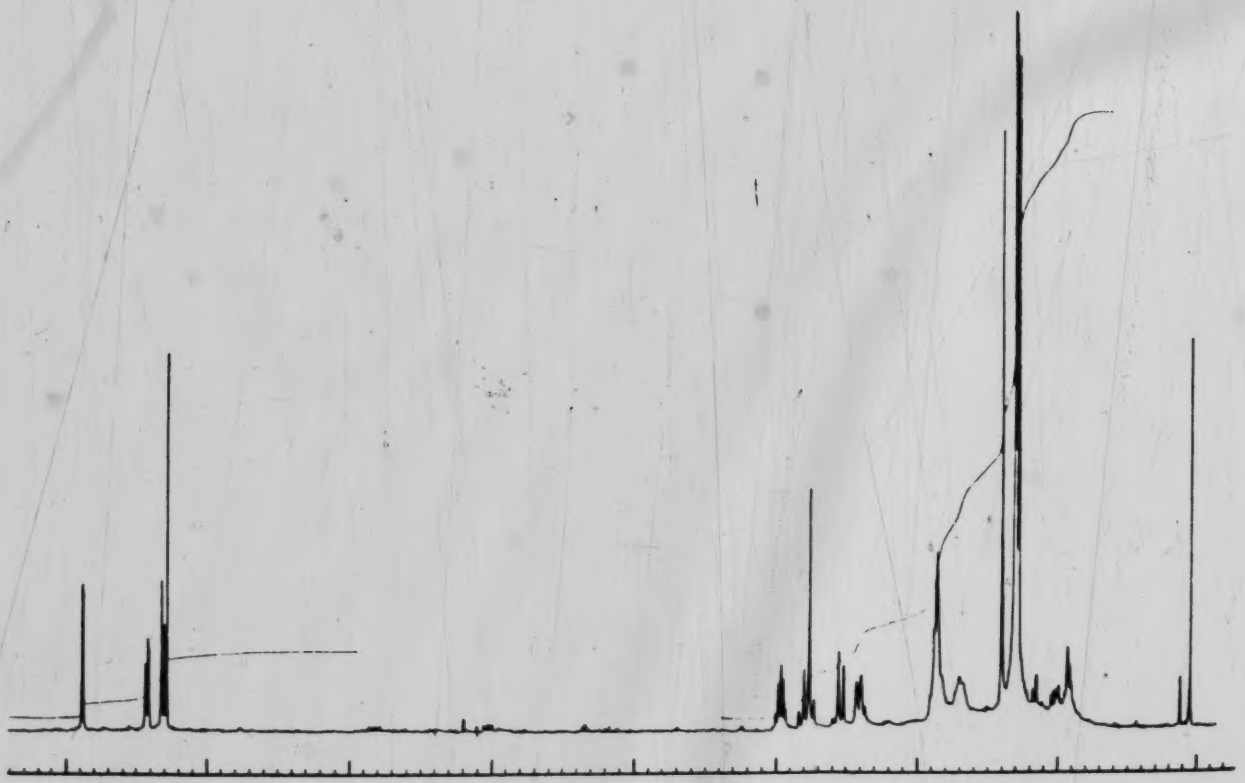


Figure 5. 400 MHz ^{1}H nmr spectrum ($CDCl_3$) of 7-oxodehydroabietic acid (43)

the ^{13}C nmr spectrum at δ198.4. In addition to the carboxyl carbonyl at 1695 cm^{-1} in the ir spectrum, an unsaturated ketone appears at 1685 cm^{-1}. The two carbonyl bands become better resolved (1727 and 1684 cm^{-1}) when the acid is esterified (diazomethane) to give 44. The uv spectrum is also in accord with an aromatic ketone (217(ε10,500), 255(ε7700), 304(ε1830)nm). Comparison of the spectroscopic data with literature values [44] for methyl-7-oxodehydroabietate (44) confirmed the identity of the parent compound 43 as 7-oxodehydroabietic acid. This compound has been reported as a natural product of Pinus sylvestris[45] and Calocedrus decurrens[46]. In the latter case, 43 was detected by gas liquid chromatography as making up 2% of the resin of C. decurrens. Several other compounds were identified, including dehydroabietic acid (major component, 17%). However, the amount of 43 was less in fresh resin than in older resin. Thus 43 may have accumulated as a result of autooxidation of 31. In fact this has been shown to occur[47]. Thus 43 isolated from from A. mellea may be an artifact.

Compound 45 was isolated from some of the most polar fractions from chromatography of the crude broth extraxct of strain NFRC-621. The identity of 7-oxo-15-hydroxy-dehydroabietic acid (45) was established from the ^{1}H nmr (Figure 6), ir, and mass spectra, and by comparison with the spectral data of 31 and 43. Thus, the three aromatic

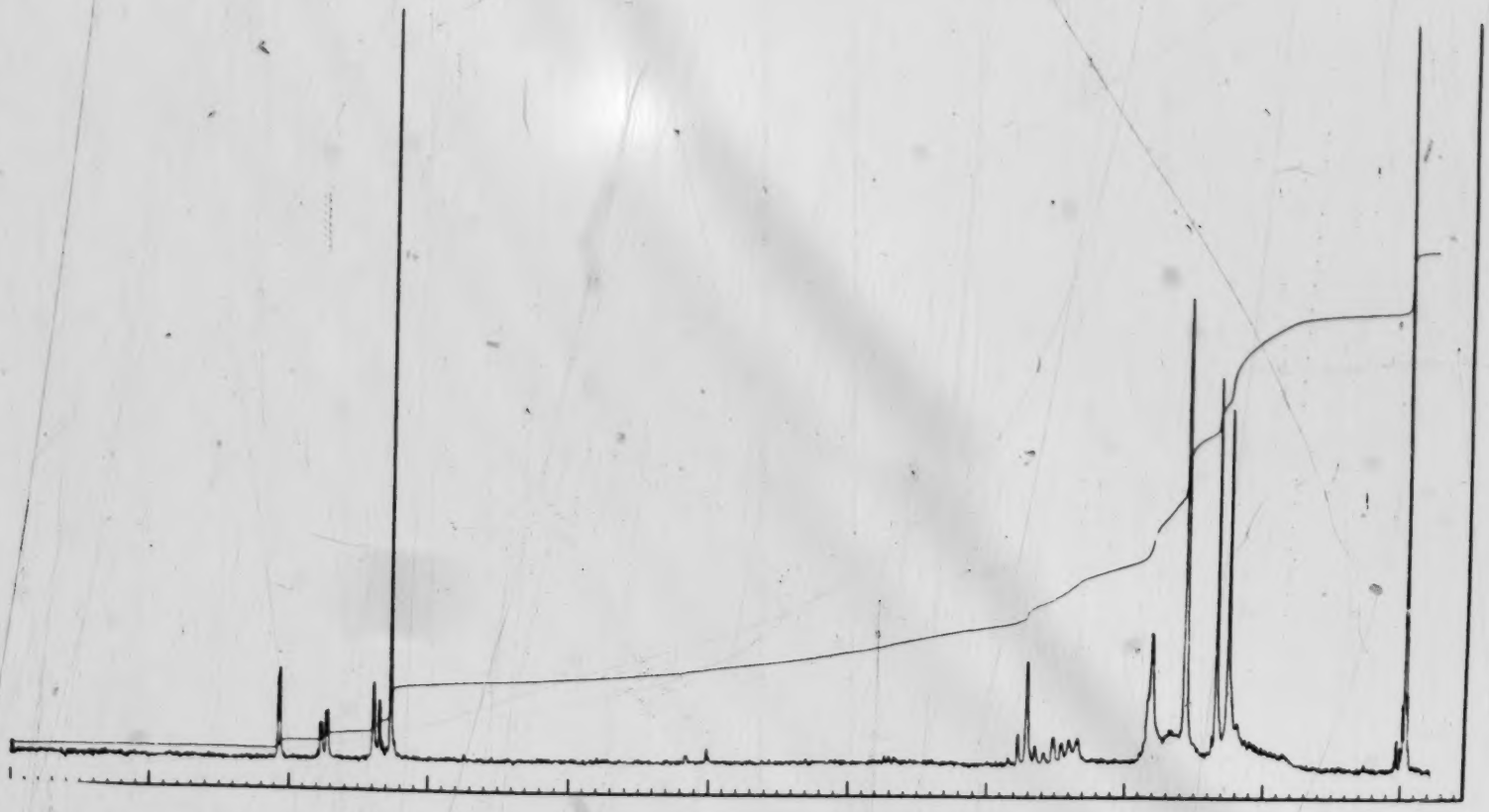
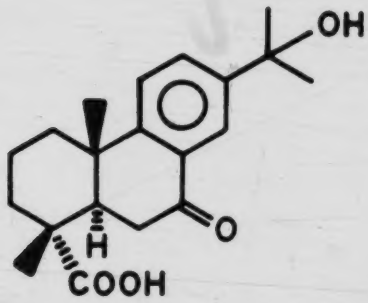


Figure 6. 200 MHz ^{1}H nmr spectrum ($CDCl_3$) of 7-oxo-15-hydroxydehydroabietic acid (45)

protons with the coupling pattern found in the C-ring of 31



45

appear again in 45. As in 43 these three protons are deshielded relative to 31. Both the C-7 methylene and benzylic isopropyl proton of 31 are absent in 45, while two methyl singlets appear at δ1.60 and δ1.61. The ketone and hydroxyl groups are thus assigned to C-7 and C-15, respectively. Although 15-hydroxy compounds are not among the reported[47] auto-oxidation products of 31, the fact that benzylic auto-oxidation does give rise to 43 suggests that 45 also may be an artifact.

On one occasion one of the strains (NFRC-631) produced orange pigments in both the broth and mycelial extracts.[3]

In this case, after preliminary chromatography of the crude broth extract using chloroform-ethyl acetate-acetic acid, 84:15:1 as solvent system, compounds 31, 34, 36,38 contained yellow material. This mixture was subjected to flash chromatography with the solvent system Skellysolve

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[3]This result was not reproducible, thus the generation of orange pigments due to contamination by another fungus cannot be ruled out.

B-ethyl acetate-acetic acid, 92:7:1. Acids 31, 34, 36, 38 eluted first, followed by two yellow bands, the first eluting when the polarity of the eluent was increased to 87:12:1, the second with 79:20:1. The quantity of the first-eluted yellow material obtained was too small for spectroscopic identification of its components (vide infra). Fractions containing the second yellow band were combined and evaporated to dryness in vacuo. The orange solid obtained was redissolved in toluene and cooled to ca. 5°C overnight, resulting in the formation of crystalline material (mp 232-234°C) which appeared to be a pure compound (46) by tlc. The molecular formula $C_{17}H_{10}O_7$ was established by high resolution mass spectroscopy. The ir spectrum shows an OH band at 3480 cm^{-1}, and absorption at 1663 and 1645 cm^{-1}. The ^1H nmr spectrum (Figure 7) shows three protons exchangeable with D_2O, two hydrogen-bonded phenolic protons which appear as singlets at δ11.80 and 12.38 and an alcoholic proton at δ2.88. A 1,2,3-trisubstituted aromatic system is apparent from the coupling of the aromatic protons at δ6.80, 6.89, and 7.59. The low field position of the latter proton indicates its location para to an electron-withdrawing substituent. Protons at δ5.73 and δ6.58 are coupled (J= 2.75 Hz). The small coupling constant and the large difference in their chemical shifts indicate they are located on a highly polarised Z-olefin. This suggeted the presence of the bis-dihydrofuran moiety, typical of the aflatoxins[48]. Such a structure also accounts

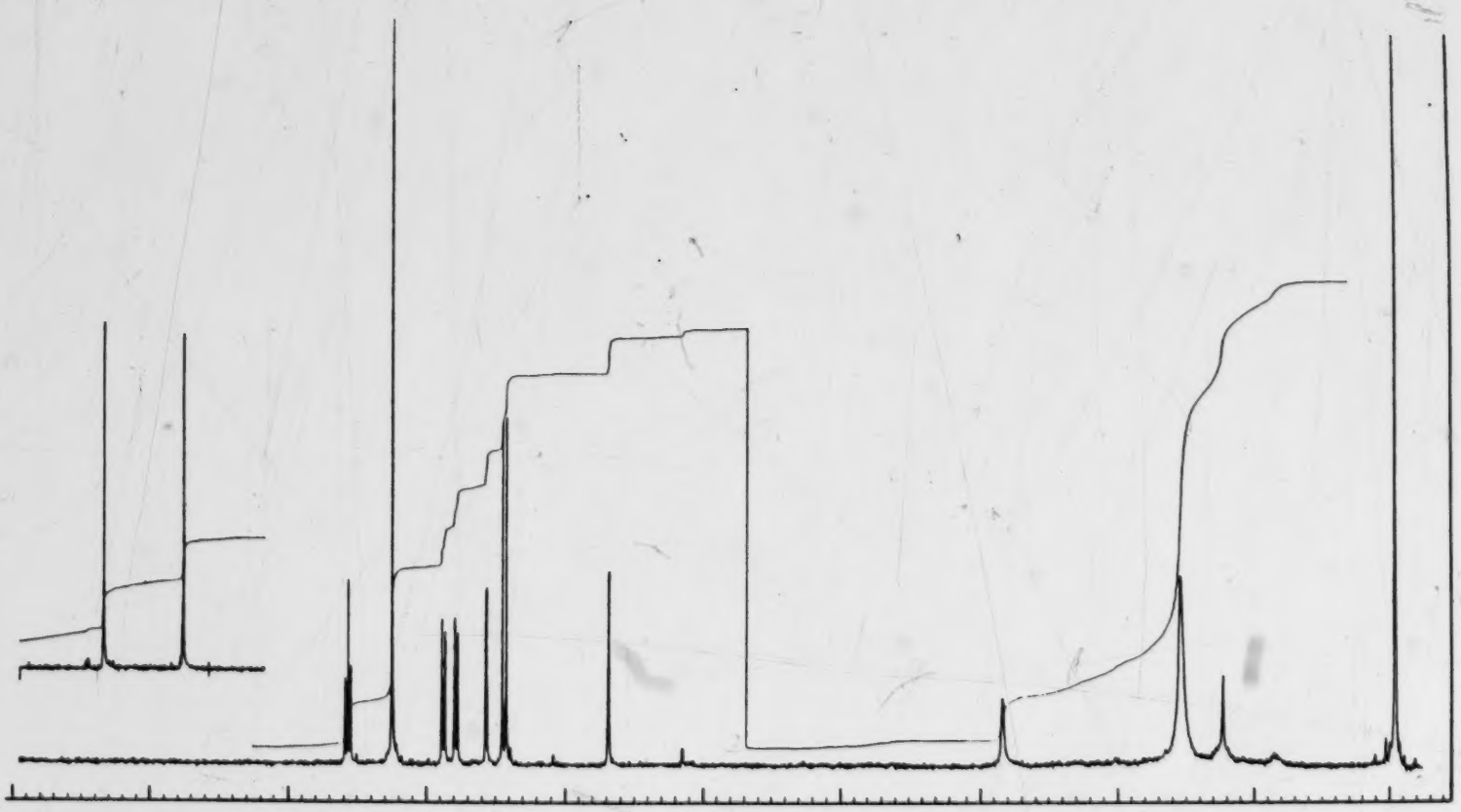
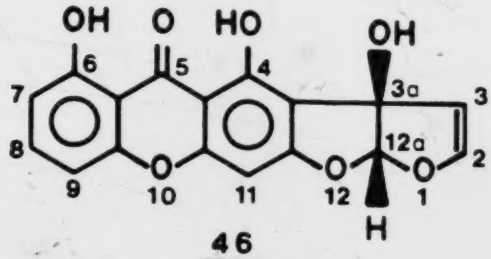


Figure 7. 400 MHz ^{1}H nmr spectrum ($CDCl_3$) of austocystin F (46)

for two low field singlets at δ6.44 and δ6.46. One is a methine deshielded by two ethereal oxygens while the other is assigned to the isolated aromatic proton at C-4. Thus the alcoholic OH must be the other ring junction substituent. The linear nature of the fused pentacyclic system follows from the chemical shift (δ6.44 or δ6.46) of the proton at C-11[49]. Comparison (tlc, ir) with an authentic sample of austocystin F confirmed the identity of the metabolite. Austocystin F has been isolated previously as a member of a series of austocystins produced by Aspergillus ustus[49].



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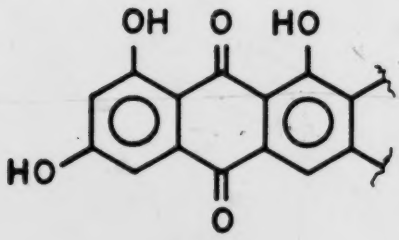
The yellow band of pigments isolated from the broth extract of strain NFRC-631 (in insufficient quantity to permit elucidation of its components) was also present in the mycelial extract of the same strain. Following flash chromatography of the crude extract, a fraction rich in orange pigments was concentrated and cooled to ca. 5°C, resulting in the formation of orange crystals. This material appeared to be the same (tlc) as the pigments obtained from the broth extract. That the orange crystals

were composed of two components was indicated by cims, which gives M+1 peaks at m/z 369 and 371. The mixture appeared homogeneous by tlc and could not be separated.[4]

In conjunction with the high resolution mass spectrum the molecular formulae were established as $C_{20}H_{16}O_7$ (368) and $C_{20}H_{18}O_7$ (370). The ir spectrum reveals an OH band (3380 cm^{-1}) as well as unsaturated carbonyl absorption (1669 and 1622 cm^{-1}). That the latter of these is a quinone carbonyl hydrogen-bonded to two peri-phenolic groups is indicated by the appearance of singlets in the ^1H nmr ($CDCl_3$) spectrum at δ12.34 (1H) and δ12.51 (1H). The ^1H nmr spectrum determined in acetone-d_6 + D_2O shows two aromatic protons, displaying mutual meta coupling (2 Hz), at δ6.58 and δ7.13, and an isolated aromatic proton at δ7.03. These data are consistent with the anthraquinone partial structure 47.



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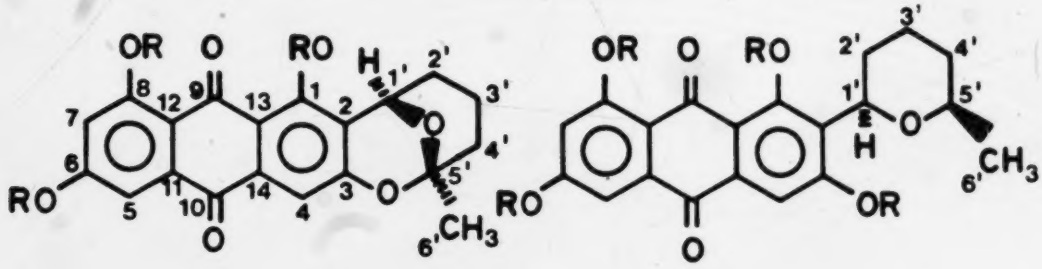
[4]In our hands, averufin and averufanin could not be separated by tlc using the method of Berger and Jadot (reference 50). Observed Rf values values were significantly lower than those reported and no separation was obtained.

Acetylation (acetic anhydride-pyridine) of the mixture afforded products which were separated by preparative tlc. Mass spectral analysis shows these to be a tetraacetate (51, $C_{28}H_{26}O_{11}$) arising from the component of molecular weight



48 R=H
49 R=$COCH_3$

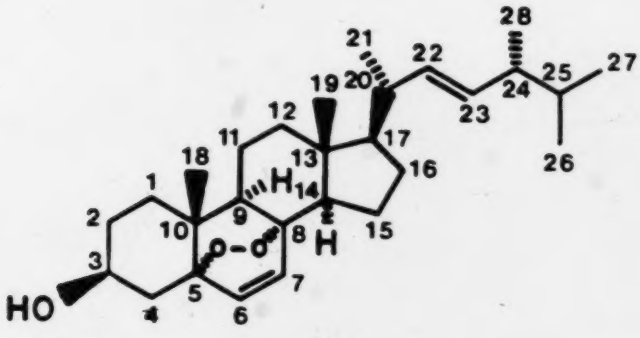
50 R=H
51 R=$COCH_3$

370, and a triacetate (49, $C_{26}H_{22}O_{10}$) formed from the compound of molecular weight 368. The ^1H nmr spectrum of the triacetate possesses a multiplet at δ5.29 (1H) and a singlet at δ1.56 (3H). These features helped establish the structure of averufin (48) as the parent compound of the triacetate. The identity was confirmed by comparison (tlc, ir, ms) with an authentic sample.

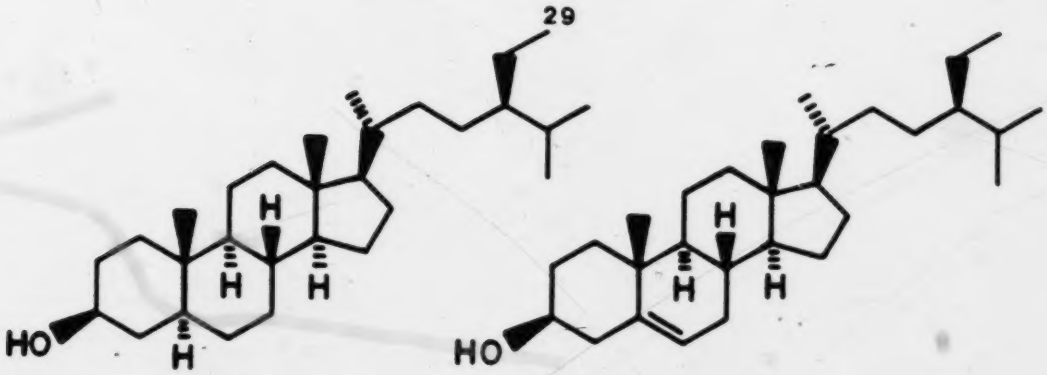
The location of a hydroxyl at C-3 in averufanin (50), the parent compound of the tetraacetate, follows from the magnitude (Δppm= 0.79) of the downfield shift of H-4 upon acetylation [51]. Such a large change can be attributed to the acetylation of both para and ortho phenols. In 48 H-4 experiences a downfield shift of only 0.41 ppm, due to acetylation of the para OH substituent. The ^{1}H nmr spectrum of 50 contains signals at δ1.31 (3H, d, J=6 Hz), 3.8 (1H, m), and 5.14 (1H, dd, J=11.5, 2.25 Hz), suggesting a methyl-substituted tetrahydropyran ring which accounts for the single remaining degree of unsaturation. Acetylation (acetic anhydride-pyridine) of an authentic sample of averufanin afforded a product identical (tlc, ir) with the tetraacetate 51 in hand.

A thorough examination of the mycelial extracts of all three strains afforded several of the steroidal compounds present, including ergosterol endo-peroxide (52), sitostanol (53), and β-sitosterol (54). Ergosterol endo-peroxide (52) was isolated from the mycelial extract of strain NFRC-621 and was purified by flash chromatography, preparative tlc, and finally recrystallisation (mp 178-181°C). The compound was identical (tlc, ir, ^{1}H nmr) with an authentic sample. Loss of a molecule of oxygen to give the base peak (m/z 302) was particularly diagnostic. The mixture of 53 and 54 (mp 136-138°C) was isolated by the same series of steps from the mycelial extract of strain NFRC-624. The presence of the saturated compound 53 in the sample of 54 was indicated by

the mass spectrum, which shows a peak of 19% intensity at m/z 416 (M^+ of 53), whereas an intensity of only 5% would be anticipated for the M+2 peak of m/z 414 (M^+ of 54)[52]. The position of the carbon-carbon double bond in 54 was deduced from the ms fragmentation pattern[53]. Compounds 53 and 54 could not be separated on tlc, even when silica gel



52

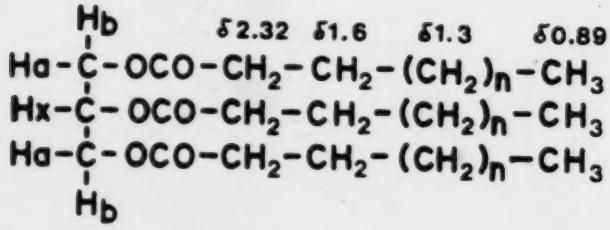


53 54

impregnated with silver nitrate was utilised. The stereochemistry at C-24 was assigned as R since the S epimer of 54 (clionasterol) has a melting point of 147-148°C[54].

From the mycelial extract of strain NFRC-631, relatively large amounts of the least polar (tlc) component were obtained as a clear liquid following flash chromatography. Examination of the ^1H nmr spectrum indicates a triglyceride structure. Thus the methyl and $(CH_2)_n$ methylene groups (Scheme 6) of the fatty acid

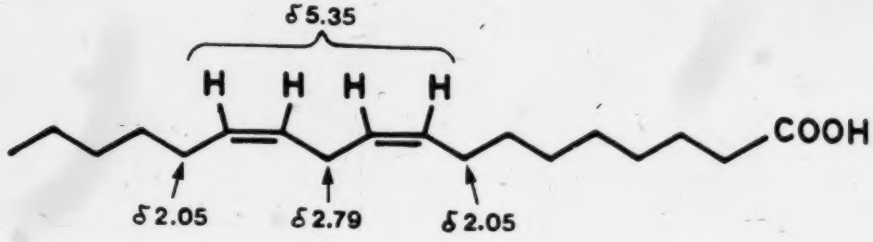
Scheme 6 **^1H nmr assignment of triglycerides**



linkages appear at δ0.89 and δ1.3, respectively. Methylene groups α and β to the carbonyl carbon give rise to signals at δ2.32 (t, J=7.5 Hz) and δ1.6 (m), respectively. Protons designated H_a and H_b are assigned to resonances at δ4.15 (dd, J=12, 6 Hz) and δ4.32 (dd, J=12, 4.5 Hz). Additional signals suggest that linoleic acid (55, vide infra) makes up part of the fatty acid content of the triglycerides. Carbonyl absorption due to the esters appears at 1745 cm^{-1}

in the ir spectrum.

Scheme 7 ^{1}H nmr assignment of linoleic acid (55)



55

Hydrolysis (10% (w/v) aqueous potassium hydroxide) of the triglycerides yielded a mixture of fatty acids (ir: 3400-2500 cm^{-1}(OH) and 1706 cm^{-1}(C=O)). Signals at δ2.05, 2.79, and 5.35 (Scheme 7) in the ^{1}H nmr spectrum indicate that the unsaturated fatty acid linoleic acid (55) makes up a significant portion of the mixture.

Saturated fatty acids were also crystallised directly from fractions obtained after flash chromatography of the mycelial extract of strain NFRC-631. A typical sample (mp 59-61°C) contained acids of 18, 20, 22, and 24 carbon atoms, as indicated by cims.

Fifteen distinct species (five in Europe and ten in North America) of the *A. mellea* complex have been identified[55]. The three strains employed in this study are "biological species I" of Anderson and Ullrich[55] or the closely related "foothills type" of Mallett[56]. They correspond to a taxonomic species *Armillaria ostoyae* (Romagn.) Herink, one of the species recognised within the *Armillaria mellea* complex in recent years[57]. Thus it is

possible that the species used in this study has not been examined previously for its metabolites, while the reported metabolites of A. mellea (vide supra, including those of Oduro) may have been isolated from one or more of the other species. Resin acids 31, 34, 36, 38, 40, 43, and 45 (and pigments 46, 48, and 50 on one occasion) were isolated when the strains used in this investigation were grown on liquid culture. These strains are parasitic on pine and spruce, themselves producers of resin acids, but the media used in this study (V-8 juice, potato dextrose) do not contain resin acids. The biological significance, if any, of this observation is not clear to us. However, it is interesting to note that in conifers large amounts of resin are exuded through the bark at the point of infection and the area of resinosis increases as the fungus spreads in the bark[2]. Although some resin acids have been shown to possess antifungal (and antibiotic) activity[58,59,60], resinosis on the part of the host tree could hardly be expected to function as an effective defense mechanism against a pathogen which metabolises the same compounds (resin acids) which constitute the resin itself. At the same time, it seems unlikely that compounds which are exuded in such large quantities by the tree could play a significant role in the pathogenic mechanism of action of the fungus on the tree. The fungus may have evolved the capacity to metabolise resin acids as a response to repetitive encounters in Nature with resin-producing coniferous trees. This capacity may no

longer have anything to do with fungus-host interactions, but perhaps is involved in the protection of the fungus from some of its predators. As stated above, some resin acids are reported to display both antifungal and antibiotic activity, and the production of chemical defense substances by A. mellea may be important as a survival factor in competition with antagonistic soil microorgsanisms[3].

Resin acids hitherto have not been reported as fungal metabolites[61].

2.2 EXPERIMENTAL

General

High resolution electron impact mass spectra (hreims) were recorded on an AEI MS-50 mass spectrometer. Unless diagnostically significant, only those peaks with intensity of >30% of the base peak are recorded. Chemical ionisation mass spectra (cims) were obtained using an AEI MS-12 mass spectrometer with ammonia as the reagent gas. The data were processed in DS-55 and Nova-4 computers. Fourier transform infrared (ftir) spectra were recorded as $CHCl_3$, CH_2Cl_2, or CH_3OH casts on a Nicolet 7199 FTIR interferometer. Single scan infrared (ir) spectra were recorded on a Perkin Elmer 297 spectrometer. Nuclear magnetic resonance (nmr) spectra (^1H and ^{13}C) were obtained on Bruker WH-200 or WH-400 spectrometers with an Aspect 2000 computer system. Tetramethylsilane was used as the internal standard. Ultraviolet (uv) spectra were obtained on a Unicam SP 1700 spectrophotometer and optical rotations on a Perkin Elmer 141 polarimeter. Melting points are uncorrected and were determined on Zeitz-Wetzlar and Thomas Model 40 melting point apparatus. Macherey Nagel silica gel 60 (less than 200 mesh) was used for column chromatography. Fractions were collected with a Buchler Fracto Mette 200 fraction collector. E. Merck silica gel 60 (230-400 mesh) was used for flash chromatography. Preparative thin layer chromatography (ptlc) was carrried out on Terochem silica

gel G containing a trace of electronic phosphor (General Electric), 20x20 cm, 10x20 cm, or 5x20 cm plates, 0.35 mm thickness. Analytical thin layer chromatography (tlc) was done on glass plates (75x25 or 75x50 mm) coated with the same adsorbent as ptlc, or on E. Merck precoated plates of silica gel 60 F-254. Analytical tlc plates with adsorbed silver nitrate ($AgNO_3$) were prepared by dipping the precoated plates into a solution of 20% (w/v) $AgNO_3$ in H_2O-CH_3OH, 1:1 (stored in the dark) for several seconds, then drying with a heat gun. The plates were used immediately. Unless otherwise indicated, R_f values are for single elutions. Ultraviolet active materials were detected by visualisation under a uv lamp (254 or 350 nm). The plate (only a thin vertical band in the case of ptlc) was then sprayed with a solution of 10% or 30% sulphuric acid and charred on a hot plate. Unless specified, all solvents, with the exception of ether, hexane, and tetrahydrofuran, were distilled prior to use. Skellysolve B refers to Skelly Oil Company light petroleum, bp 62-70°C.

Fungal Strains

The strains of Armillaria mellea used in this study were obtained from Dr. Y. Hiratsuka, Northern Forest Research Centre (NFRC), Edmonton. They were collected from the following host trees in British Columbia: strain NFRC-621 (modified A. bulbosa rhizomorphs) from lodgepole pine; strain NFRC-624 (A. mellea rhizomorphs) from ponderosa

pine; strain NFRC-631 (no rhizomorphs) from white spruce. The rhizomorph types were determined by Dr. K. Mallett.

Slant tube cultures of these strains were transferred to potato dextrose agar (PDA) slant tubes which were kept as stock cultures at 4°C.

To initiate still cultures of the fungus, small fragments of agar containing the mycelium were aseptically transferred to Erlenmeyer flasks (500 mL, one or two per strain), each containing about 200 mL of sterile culture medium (a 10% (v/v) solution of clarified V-8 juice containing 1% (w/v) glucose). The flasks were stoppered with foam plugs covered loosely with aluminum foil. The cultures were allowed to mature in a darkened, well-ventilated area without agitation. White mycelium and rhizomorphs appeared, both of which gradually became brown in colour. After two weeks incubation, the inoculum from each strain was blended (Waring blender, sterilised in autoclave) and 10-20 mL aliquots were aseptically transferred to each of 8-10, 1 litre Fernbach flasks charged with sterile medium (either a 10% (v/v) solution of clarified V-8 juice containing 1% (w/v) glucose, or potato dextrose broth (PDB)). The flasks were stoppered with foam plugs covered loosely with aluminum foil. Growth periods were from 34 to 92 days.

Extraction of the Metabolites

The culture mixture was separated into broth and mycelium by filtration through cheese cloth. The culture broth was extracted with chloroform (ca. equal volume). The chloroform extracts contained emulsions, and vacuum filtration through Celite facilitated the separation of organic and aqueous phases. The chloroform was dried over sodium sulphate and evaporated in vacuo to afford the crude metabolites. Although yields of crude metabolites varied with different growth periods, the resin acids (vide infra) were produced consistently by all three strains. The mycelium was extracted with chloroform or methanol in a Soxhlet extractor. The organic extract was dried over sodium sulphate and concentrated to dryness in vacuo.

Authentic Samples

Authentic samples were kindly provided by Dr. O.E. Edwards and Dr. E. Wenkert (pimaric (34), isopimaric (36), and sandaracopimaric (38) acids; Dr. R. Vleggaar (austocystin F (46); Dr. J.C. Vederas (averufin (48; and Dr. A. Castonguay (averufanin (50).

Isolation of dehydroabietic acid (31), pimaric acid (34), isopimaric acid (36), and sandaracopimaric acid (38)

The crude broth metabolites of strain NFRC-621 (1.8 g) were subjected to flash chromatography (chloroform-ethyl aceate-acetic acid, 94:5:1) to afford 0.547 g of a mixture of compounds 31, 34, 36, and 38. A 40 mg sample of the

mixture was applied to a 20x20 cm ptlc plate and developed twelve times with Skellysolve B-ethyl acetate-acetic acid, 98:2:1. Careful scraping of the lower edge of the band corresponding to the mixture, and elution from the silica gel yielded 3.2 mg of impure 31. This sample was further purified by ptlc on a 5x20 cm plate using the same solvent system as above and developing five times, followed by scraping and elution to give 31, pure as judged by multiple-development tlc on silver nitrate-impregnated silica gel, using both protic and aprotic solvent systems.

A 90 mg sample of the mixture (31, 34, 36, 38) was chromatographed on 10 g silica gel with 2 g adsorbed silver nitrate. The silica gel and powdered silver nitrate were mixed by combining with an organic solvent to create a slurry, then removing the solvent *in vacuo* on a rotary evaporation apparatus. The column (1.5 cm diameter) was wrapped in aluminum foil. Fractions (5 mL) were collected from elution with chloroform-Skellysolve B, 50:50, then 75:25 (each for approximately one column volume), then 100% chloroform to fraction 175, and finally chloroform-ethyl acetate, 98:2 to fraction 260.

Fractions 30-69 gave 31 mg of impure 31. Further purification by flash chromatography (chloroform-ethyl acetate, 98:2) afforded 19 mg of 31, mp 162-168°C (CH_3OH-H_2O)(lit.[37] mp 171-172°C); $[\alpha]_D$ +54° (*c* 1.9 95% EtOH)(lit.[37] $[\alpha]_D^{25}$ +61° 95% EtOH); tlc: R_f 0.6 (Skellysolve B-ethyl acetate-acetic acid, 75:25:1), R_f 0.72

(chloroform-ethyl acetate-acetic acid, 97:2:1, 3x, silver nitrate-impregnated silica gel); ftir(CH_2Cl_2 cast): 3200-2400(b), 2950, 2925, 2865, 1692 cm^{-1}; uv(CH_3OH): 220(ϵ2180), 268(ϵ630), 276(ϵ600)nm; hreims: m/z. calcd. for $C_{20}H_{28}O_2(M^+)$: 300.2089: found 300.2093(30), 285(100), 239(74); ^1H nmr($CDCl_3$): δ1.0-1.9(7H, m), 1.23(6H, d, J=7 Hz, C-15 2xCH_3), 1.25(3H, s, C-10 CH_3), 1.29(3H, s, C-4 CH_3), 2.24(1H, dd, J=12.5, 2 Hz), 2.30(1H, bd, J=12.5 Hz), 2.82(1H, septet, J=7 Hz, C-15H), 2.90(2H, m, C-7 CH_2), 6.87(1H, d, J=1.75 Hz, C-14 H), 6.98(1H, dd, J=8, 1.75 Hz, C-12 H), 7.14(1H, d, J=8 Hz, C-11 H); ^{13}C nmr($CDCl_3$): δ16.3, 24.0 (2x), 25.2(CH_3); 18.6, 21.9, 30.1, 36.9, 38.1(CH_2); 33.5, 44.7, 124.0, 124.2, 127.0(CH); 37.0, 47.5, 134.8, 145.8, 146.9(C); 185.0(C=O).

Fractions 95-169 afforded 3.3 mg of 34, mp 200-208°C (95% EtOH)(lit.[62] mp 212°C); tlc: R_f 0.65 (chloroform-ethyl acetate-acetic acid, 97:2:1, 3x, silver nitrate-impregnated silica gel); ftir($CHCl_3$ cast): 3600-2400(b), 2925, 2870, 2850, 1695 cm^1; hreims: m/z calcd. for $C_{20}H_{30}O_2(M^+)$: 302.2246; found 302.2243(49), 287(69), 167(37), 121(100), 91(31); ^1H nmr($CDCl_3$): see Table 1; δ0.80(3H, s, C-10 CH_3), 0.8-1.8(13H, m), 1.02(3H, s, C-13 CH_3), 1.23(3H, s, C-4 CH_3), 1.95(1H, dd, J=12.5, 2.5 Hz), 2.13(1H, m), 2.29(1H, m); ^{13}C nmr($CDCl_3$): δ15.0, 16.7, 29.4(CH_3); 18.0, 18.9, 25.1, 35.3, 35.6, 37.0, 38.1, 116.4(CH_2); 48.7, 51.4, 128.4, 147.2(CH); 37.8, 38.6, 47.3, 137.9(C); 185.8(C=O).

The later fractions gave 40 mg of impure 36 and 38. After this sample was subjected twice more to flash chromatography (Skellysolve B-ethyl acetate-acetic acid, 95:5:1 and chloroform-ethyl acetate, 90:10, respectively) a trace of 34 was still present by tlc (silver nitrate). The remainder (17 mg) was subjected to flash chromatography (chloroform-ethyl acetate-acetic acid, 97:2:1) on silica gel (2.4 g) thoroughly mixed with powdered silver nitrate (0.6 g) to give 5 mg of 36 and 38, appearing as a single spot by tlc: R_f 0.52 (chloroform-ethyl acetate-acetic acid, 97:2:1, 3x, silver nitrate-impregnated silica gel); hreims: m/z calcd. for for $C_{20}H_{30}O_2(M^+)$: 302.2246; found 302.2250(100), 287(47), 273(29), 257(27), 241(34), 187(30), 133(20), 121(19), 119(26), 105(31), 93(18), 91(33); ^1H nmr($CDCl_3$): see Table 1; δ0.85(s, C-10 CH_3 of 38), 0.87(s, C-10 CH_3 of 36), 0.91(s, C-13 CH_3 of 36), 1.05(s, C-13 CH_3 of 38), 1.1-2.0(m), 1.22(s, C-4 CH_3 of 38), 1.28(2, C-4 CH_3 of 36).

Esterification of 31, 34, 36, and 38

An excess of diazomethane in ether was added to a magnetically stirred solution of the mixture of acids (28 mg; tlc: R_f 0.67 (Skellysolve B-ethyl acetate-acetic acid, 75:25:1); ir($CHCl_3$): 3400-2400(b), 1690 cm^{-1}) dissolved in ether (1 mL). Evaporation to dryness gave a colourless oil (28 mg); tlc: R_f 0.82 (Skellysolve B-ethyl acetate-acetic acid, 75:25:1); ir($CHCl_3$): 1720 cm^{-1}; cims: m/z 334(M+18 for esters 35, 37, 39), 332(M+18 for ester 32), 317(M+1 for

esters 35, 37, 39), 315(M+1 for ester 32).

Similarly 2.5 mg of purified 34 was esterified to give methyl pimarate (35); tlc: R_f 0.67 (chloroform-ethyl acetate-acetic acid, 97:2:1, 2x, silver nitrate-impregnated silica gel); ftir($CHCl_3$ cast): 2918, 2849, 1728, 1244, 914 cm^{-1}.

Chromatography of the methyl esters 32, 35, 37, and 39

The mixture of esters (47 mg) was subjected to column chromatography over neutral alumina (36.4 g; Woelm, ICN Pharmaceuticals, Inc.) mixed thoroughly with powdered silver nitrate (7.3 g). The column (1.5 cm diameter) was wrapped in aluminum foil and eluted with hexane (7 fractions, 8 mL), hexane-ether, 98:2 (to fraction 38), and finally hexane-ether, 95:5. Methyl dehydroabietate (32) and methyl pimarate (35) eluted together, methyl isopimarate and methyl sandaracopimarate (6 mg; 37 and 39) eluted in fractions 67-88; tlc: R_f 0.44 (Skellysolve B-ethyl acetate, 90:10, silver nitrate-impregnated silica gel); ftir($CHCl_3$ cast): 3080(w), 2950, 2920, 2865, 2845, 1728(s), 1637(w), 1244, 909 cm^{-1}; cims: m/z 334(M+18); ^1H nmr($CDCl_3$): δ0.83(s), 0.86(s), 0.90(s), 1.04(s), 1.1-2.0(m), 1.20(s), 1.26(s), 3.665(s), 3.67(s), 4.86(dd, J=10.5, 1.5 Hz), 4.88(dd, J=10.5, 1.5 Hz), 4.90(dd, J=17.25, 1.5 Hz), 4.92(dd, J=17.25, 1.5 Hz), 5.21(bs), 5.31(dd, J=7.5, 1.5 Hz), 5.77(dd, J=17.25, 10.5 Hz), 5.80(dd, J=17.25, 10.5 Hz).

Hydrogenation of 31, 34, 36, and 38

The mixture of acids (10 mg) was dissolved in dry ethyl acetate (9 mL, dried by passing through a column packed with sodium sulphate) and activated 10% palladium on charcoal (102 mg) was added. The mixture was pressurised with hydrogen (15 psi) in a Parr hydrogenator for 24 hours at room temperature. The catalyst was removed by filtration through Celite. Evaporation of the filtrate in vacuo afforded 10 mg of a colourless solid; tlc: R_f 0.56 (Skellysolve B-ethyl acetate-acetic acid, 75:25:1); ir($CHCl_3$ cast): 3400-2400(b), 1695(s) cm^{-1}; cims: m/z 324(M+18 for hydrogenation products of 34, 36, and 38), 318(M+18 for unchanged 31).

Isolation of levopimaric acid endo-peroxide (40)

The crude broth metabolites of strain NFRC-631 (0.74 g) were subjected to flash chromatography (chloroform-ethyl acetate, 90:10 to fraction 16, then chloroform-ethyl acetate-acetic acid, 97:2:1 to fraction 21, and 94:5:1 to fraction 25). Elution was continued with Skellysolve B-ethyl acetate, 75:25 (100 mL), 50:50 (100 mL), and 100% ethyl acetate (100 mL). A 389 mg fraction (eluting with chloroform-ethyl acetate-acetic acid, 97:2:1 after 31, 34, 36, 38) contained 40 and was further purified by flash chromatography (chloroform-ethyl acetate-acetic acid, 97:2:1) to give 40 (269 mg); tlc: R_f 0.43 (chloroform-acetone-acetic acid, 89:10:1, 5x); ftir($CHCl_3$

cast): 3600-2400(b), 2955, 2935, 2870, 1692 cm^{-1}; uv(CH_3OH): 274(sh)(ϵ340); hreims: m/z calcd. for $C_{20}H_{30}O_4(M^+)$: 334.2144; found 334.2149(18), 319(14), 316(18), 302(100), 291(18), 285(21), 275(25), 245(51), 146(37), 121(56), 113(32), 107(32), 105(38), 93(38), 91(58); ^1H nmr($CDCl_3$): δ0.56(3H, s, C-10 CH_3), 0.9-2.4(14H, m), 1.08 and 1.12(2x 3H, d, J=7 Hz, C-15 2x CH_3), 1.15(3H, s, C-4 CH_3), 2.50(1H, septet, J=7 Hz, C-15 H), 4.61(1H, m, C-12 H), 5.87(1H, s, C-14 H), 9.0(1H, bs, COOH); ^{13}C nmr($CDCl_3$): δ15.1, 16.2, 20.1, 20.3(CH_3); 16.9, 21.6, 24.9, 32.0, 36.1, 36.6(CH_2); 31.0, 48.8, 50.0, 74.6, 124.6(CH); 36.8, 46.8, 76.9, 149.0(C); 184.9(C=O).

Esterification of levopimaric acid endo-peroxide (40)

Treatment of 40 (12 mg) with excess diazomethane in ether overnight at room temperature yielded 12 mg of methyl ester 41 upon evaporation to dryness; tlc: R_f 0.56 (chloroform-acetone-acetic acid, 89:10:1, 5x); ir($CHCl_3$): 2950, 2875, 1720, 1300-1190(b) cm^{-1}; hreims: m/z calcd. for $C_{21}H_{32}O_4(M^+)$: 348.2300; found 348.2302(9), 333(5), 316(100), 146(48), 121(70), 91(36); ^1H nmr($CDCl_3$): δ0.58(3H, s), 0.9-2.4(14 H, m), 1.10(3H, d, J=7 Hz), 1.14(3H, d, J=7 Hz), 1.17(3H, s), 2.51(1H, septet), 3.70(3H, s), 4.62(1H, m), 5.89(1H, s).

Isolation of 7-oxodehydroabietic acid (43)

Flash chromatography of the crude broth metabolites of strain NFRC-621 (vide supra) afforded impure 43 in fractions eluting with chloroform-ethyl acetate-acetic acid, 94:5:1 after the fractions containing the mixture of 31, 34, 36, and 38. This material (153 mg) was further subjected to flash chromatography (chloroform-ethyl acetate, 94:6 containing 3 drops acetic acid per 10 mL) to give impure 43 (37 mg). Preparative tlc (chloroform-ethyl acetate, 90:10, 5x) yielded, after careful scraping and elution, 12 mg of 43. $[\alpha]_D$ -3° (c 1.56 95% EtOH); tlc: R_f 0.23 (Skellysolve B-ethyl acetate-acetic acid, 85:15:1); ftir($CHCl_3$ cast): 3500-2400(b), 2955, 2930, 2870, 1695, 1685 cm^{-1}; uv(CH_3OH): 217(ϵ10,500), 255(ϵ7700), 304(ϵ1830)nm; hreims: m/z calcd. for $C_{20}H_{26}O_3(M^+)$: 314.1882; found 314.1883(58), 299(35), 253(100), 211(26); ^1H nmr($CDCl_3$): δ0.8-1.9(4H, m), 1.234 and 1.237(2x 3H, d, J=7 Hz, C-15 2x CH_3), 1.26(3H, s, C-4 CH_3), 1.34(3H, 3H, s, C-10 CH_3), 2.36(2H, bd, J=13 Hz), 2.49 and 2.70(2x 1H, d, J=14 Hz, C-6 H_{eq} and C-5 H), 2.76(1H, dd, J=14, 14 Hz, C-6 H_{ax}), 2.92(1H, septet, J=7 Hz, C-15 H), 7.29(1H, d, J=8 Hz, C-11 H), 7.41(1H, dd, J=8, 2 Hz, C-12 H), 7.87(1H, d, J=2 Hz, C-11 H); ^{13}C nmr($CDCl_3$): δ16.2, 23.65, 23.69, 23.76(CH_3); 18.2, 36.7, 37.2, 37.8(CH_2); 33.6, 43.8, 123.4, 125.3, 132.5(CH); 29.7, 46.4, 130.9, 147.0, 153.0(C); 182.1, 198.4(C=O).

Esterification of 7-oxodehydroabietic acid (43)

An excess of diazomethane in ether was added to a magnetically stirred solution of 43 (4.5 mg) in $CHCl_3$ (2 mL) and the reaction was stirred for 15 minutes at room temperature. Evaporation to dryness afforded 4.5 mg of methyl ester 44; $[\alpha]_D$ +2° (c 4.5 95% EtOH)(lit.[44] $[\alpha]_D$ +7.9° (EtOH)); tlc: R_f 0.41 (Skellysolve B-ethyl acetate-acetic acid, 85:15:1); ftir($CHCl_3$ cast): 2955, 2936, 2868, 1727(s), 1684(s), 1608(w), 1250 cm^{-1}; hreims: m/z calcd. for $C_{21}H_{28}O_3(M^+)$: 328.3038; found 328.2037(66), 313(6), 253(100); ^1H nmr($CDCl_3$): δ0.8-1.9(5H, m), 1.255(6H, d, J=7 Hz, C-15 2x CH_3), 1.27(3H, s, C-4 CH_3), 1.35(3H, s, C-10 CH_3), 2.36(2H, m), 2.73(2H, m), 2.93(1H, septet, J=7 Hz, C-15 H9, 3.66(3H, s, OCH_3), 7.29(1H, d, J=8 Hz, C-11 H), 7.40(1H, dd, J=8, 2 Hz, C-12 H), 7.87(1H, d, J=2 Hz, C-14 H).

Isolation of 7-oxo-15-hydroxydehydroabietic acid (45)

The polarity of the eluent in the flash chromatography of the crude broth metabolites of strain NFRC-621 (vide supra) was increased to chloroform-ethyl acetate-acetic acid, 84:15:1 after 31 fractions. A polar fraction (201 mg) containing 45 was further subjected to flash chromatography (sample dissolved in chloroform; Skellysolve B-ethyl acetate-acetic acid, 70:30:1 to fraction 18, then 65:35:1). Fractions 29-34 gave 12 mg of material which was further purified by flash chromatography (chloroform-acetone-acetic acid, 90:10:1) to afford 45 (3 mg). tlc: R_f 0.4

(chloroform-acetone-acetic acid, 89:10:1, 3x); ftir($CHCl_3$ cast): 3600-2400(b), 2970, 2930, 2867, 1696(b), 1237, 756 cm^{-1}; hreims: m/z calcd. for $C_{20}H_{26}O_4(M^+)$: 330.1831; found 330.1826(3), 315(100), 312(3), 269(8); ^1H nmr($CDCl_3$): δ0.8-1.9(5H, m), 1.29(3H, s, C-10 CH_3), 1.37(3H, s, C-4 CH_3), 1.60 and 1.61(2x 3H, s, C-15 2x CH_3), 2.38(2H, bd, J=11 Hz), 2.49 and 2.58(2x 1H, d, J=12.5 and 12.25 Hz respectively, C-6 H_{eq} and C-5 H), 2.74(1H, dd, J= 12.5, 12.25 Hz, C-6 H_{ax}), 7.39(1H, d, J=8.5 Hz, C-11 H), 7.77(1H, dd, J=8.5, 2.25 Hz, C-12 H), 8.09(1H, d, J=2.25 Hz, C-14 H).

Isolation of austocystin F (46)

Flash chromatography of the crude broth metabolites of strain NFRC-631 (vide supra) gave 109 mg of material containing 31, 34, 36, and 38 as well as 46, 48, and 50 in fractions eluting with chloroform-ethyl acetate-acetic acid. This sample was further purified by flash chromatography (sample dissolved in chlorofrom; Skellysolve B-ethyl acetate-acetic acid, 92:7:1) to afford the mixture of acids, then Skellysolve B-ethyl acetate-acetic acid, 87:12:1 co-eluted 48 and 50, and Skellysolve B-ethyl acetate-acetic acid, 79:20:1 gave 46. Recrystallisation from toluene provided 46 (2 mg, mp 232-234°C, lit.[49] mp 230-233°C); $[\alpha]_D$ -180° (c 1.1 pyridine)(lit.[49] $[\alpha]_D^{22}$ -244° (c 0.91 pyridine); tlc: R_f 0.35 (Skellysolve B-ethyl acetate-acetic acid, 85:15:1,3x); ftir(CH_2Cl_2 cast): 3480(b), 1663, 1645, 1606, 1491 cm^{-1}; hreims: m/z calcd. for $C_{17}H_{10}O_7(M^+)$:

326.0426; found 326.0423(58), 297(100), 271(15); ^{1}H nmr($CDCl_3$): δ2.88(1H, bs, C-3a OH), 5.73(1H, d, J=2.75 Hz, C-3 H), 6.44 and 6.46(2x 1H, s, C-11 H and C-12a H), 6.58(1H, d, J=2.75 Hz, C-2 H), 6.80(1H, d, J=8.25 Hz, C-7 H), 6.89(1H, d, J=8.25 Hz, C-9 H), 7.59(1H, t, J=8.25 Hz, C-8 H), 11.80(1H, s, C-4 OH), 12.38(1H, s, C-6 OH).

Isolation of averufin (48) and averufanin (50)

The amounts of 48 and 50 from the broth extract of strain NFRC-631 (vide supra) were too small for identification purposes. Soxhlet extraction (chloroform) of the mycelium of the same growth of NFRC-631 mentioned above provided 7.2 g of crude metabolites. A portion (3.3 g) was subjected to flash chromatography (sample dissolved in chloroform), eluent (Skellysolve B-ethyl acetate, 93:7(750 mL), then Skellysolve B-ethyl acetate, 85:15(750 mL), then chloroform-methanol, 99:1(500 mL), then chloroform-methanol, 98:2(750 mL) and finally chloroform-methanol, 95:5(500 mL). Fraction 24 (eluting with Skellysolve B-ethyl acetate, 85:15) afforded orange crystals upon concentration and cooling to ca. 5°C. The crystalline material (23 mg) was a mixture of 48 and 50. tlc: R_f 0.50 (Skellysolve B-ethyl acetate-acetic acid, 85:15:1, 3x); R_f 0.20 (chloroform-ethyl acetate, 98:2, 3x); ftir($CHCl_3$ cast): 3380(b), 1669(w), 1622, 1399, 1268, 1258, 1156, 751 cm^{-1}; hreims: m/z calcd. for $C_{20}H_{16}O_7$(M^+ for 50: 370.1052; found 370.1040(17); m/z calcd. for $C_{20}H_{16}O_7$(M^+ for 48: 368.0896; found 368.0891(57),

350(22), 325(89), 310(100), 297(71), 286(58);cims: m/z 386(M+18 for 48), 371(M+1 for 50), 369(M+1 for 48); ^{1}H nmr(acetone-d_6+D_2O): δ1.1-2.1(m), 1.24(<3H, d, J=6.5 Hz, C-5' CH_3 of 50), 1.49(<3H, s, C-5' CH_3 of 48), 3.73(<1H, m, C-5' H of 50), 5.04(<1H, dd, J=11.5, 2.25 Hz, C-1' H of 50), 5.22(<1H, d, J=2.75 Hz, C-1' H of 48), 6.58(1H, d, J= 2 Hz, C-7 H), 7.03(1H, s, C-4 H), 7.13(1H, d, J=2 Hz, C-5 H).

Acetylation of averufin (48) and averufanin (50)

Acetic anhydride (1.0 mL) and pyridine (1.0 mL) were added to 48 and 50 (3.5 mg) and the reaction mixture was magnetically stirred for 18 hours at room temperature. The solvents were evaporated off in vacuo and the crude products were separated by preparative tlc (chloroform-methanol, 99:1) to give averufin triacetate (49, 1.3 mg), mp 205-210°C (Skellysolve B-benzene)(lit.[63] mp 210-214°C); $[\alpha]_D$ -25° (c 2.2 $CHCl_3$)(lit.[63] optical rotation -15° $CHCl_3$); tlc: R_f 0.45 (chloroform-methanol, 99:1, 2x); ftir(CH_2Cl_2 cast): 1770, 1677, 1662, 1600, 1346, 1325, 1197 cm^{-1}; hreims: m/z calcd. for $C_{26}H_{22}O_{10}(M^+)$: 494.1213; found 494.1211(10), 452(61), 410(100), 368(49), 352(84), 340(32), 325(77), 310(99), 309(56), 297(69), 286(34), 285(41), 281(37); ^{1}H nmr(acetone-d_6): δ1.6-2.2(6H, m), 1.56(3H, s, C-5' CH_3), 2.37, 2.40, and 2.44(3x 3H, s, 3x $COCH_3$), 5.29(1H, m, C-1' H), 7.39(1H, d, J=3 Hz, C-7 H), 7.51(1H, s, C-4 H), 7.93(1H, d, J=3 Hz, C-5 H).

Averufanin tetraacetate (51) was also isolated (0.5 mg), tlc: R_f 0.29 (chloroform-methanol, 99:1, 2x); ftir($CHCl_3$ cast): 2920, 2850, 1776, 1677, 1595, 1367, 1324, 1183 cm^{-1}; hreims: m/z calcd. for $C_{28}H_{26}O_{11}(M^+)$: 538.1475; found 538.1475(8), 496(42), 478(24), 454(73), 436(11), 412(76), 394(28), 352(42), 300(27), 299(38), 297(31), 272(50), 57(100); ^1H nmr(acetone-d_6): δ1.15(3H, d, J=6 Hz, C-5' CH_3), 1.6-2.3(6H, m), 2.37, 2.40, 2.41, and 2.44(4x 3H, s, 4x $COCH_3$), 3.8(1H, m, C-5' H), 4.93(1H, m, C-1' H), 7.41(1H, d, J=2.25 Hz, C-7 H), 7.89(1H, s, C-4 H), 7.94(1H, d, J=2.25 Hz, C-5 H).

Averufanin tetraacetate was prepared from authentic averufanin for comparative purposes, using the same procedure described for the mixture of 48 and 50, and proved to be identical with the derivative prepared from the isolated 50.

Isolation of ergosterol endo-peroxide (52)

The crude mycelial extract (0.94 g) from strain NFRC-621 was subjected to flash chromatography. Elution with chloroform gave a fraction (86 mg) which was further chromatographed (Skellysolve B-ethyl acetate, 85:15; changed to 75:25 after 11 fractions) to provide impure 52 (16 mg). Preparative tlc (chloroform-methanol, 97:3, 3x) afforded 52 (5 mg) which was recrystallised from ether-hexane, mp 178-181°C (lit.[64] mp 181.5-183°C); tlc: R_f 0.21 (Skellysolve B-ethyl acetate, 75:25, 3x); ftir($CHCl_3$ cast): 3500-3300(b),

2960, 2870, 1458, 1375, 968 cm^{-1}; ^1H nmr($CDCl_3$): δ0.7-2.2(21H, m), 0.83(3H, s, C-10 CH_3), 0.84(6H, d, J=6.5 Hz, C-25 2x CH_3), 0.90(3H,s, C-13 CH_3), 0.91(3H, d, J=6.5 Hz, C-24 CH_3), 1.01(3H, d, J=6.5 Hz, C-20 CH_3), 3.97(1H, m, C-3 H), 5.18(2H, m, C-22 H and C-23 H), 6.23 and 6.50(2x 1H, 2x d, J=8.5 Hz, C-6 H and C-7 H).

Analysis of other mycelial extracts by tlc showed 52 to be produced by strains NFRC-624 and NFRC-631 as well. Although not isolated in pure form, ergosterol (1) was also shown to be present in the mycelial extracts of NFRC-624 by comparative tlc with an authentic sample.

Isolation of sitostanol (53) and β-sitosterol (54)

Flash chromatography of the crude mycelial extract of strain NFRC-624 (0.82 g) afforded 30 mg from fractions eluting with Skellysolve B-ethyl acetate (75:25). Preparative tlc of this material (Skellysolve B-ethyl acetate, 85:15, 4x) yielded a mixture of 53 and 54 (6 mg), which was recrystallised from methanol-ether(mp 136-138°C)(lit.[65] mp 136-137°C); tlc: R_f 0.37 (Skellysolve B-ethyl acetate, 85:15, 3x); ftir($CHCl_3$ cast): 3600-3100(b), 2955, 2930, 2865, 1465, 1375, 1057 cm^{-1}; hreims: m/z calcd. for $C_{29}H_{52}O$(M^+ for 53): 416.4018; found 416.4005(19), m/z calcd. for $C_{29}H_{50}O$(M^+ for 54): 414.3861; found 414.3871(73), 401(6), 399(19), 396(30), 303(32), 159(30), 145(40), 133(32), 121(33), 119(33), 109(33), 107(62), 105(41),

95(67), 93(46), 91(36), 81(74), 69(62), 57(82), 55(100); ^{1}H nmr($CDCl_3$): δ0.66 and 0.69(2x s, C-10 of 53 amd 54), 0.7-2.4(m), 0.81-0.87(C-13 of CH_3 of 53, C-25 2x CH_3, and C-28 CH_3 of 53 and 54), 0.90-0.95(2x d, J= 7 Hz, C-20 CH_3 of 53 and 54), 1.03(s, C-13 CH_3 of 54), 3.52(bs, C-3 H of 53 and 54), 5.38(m, C-6 H of 53).

Isolation of fatty acids and triglycerides

Flash chromatography of the crude mycelial extracts of strain NFRC-631 (see isolation of 48 and 50) afforded crude triglycerides (531 mg) in fractions 4-5. These were further purified by flash chromatography (Skellysolve B-ethyl acetate, 95:5) to give mixed triglycerides (370 mg) as a colourless liquid, tlc: R_f 0.68 (Skellysolve B-ethyl acetate-acetic acid, 85:15:1, 2x); ftir($CHCl_3$ cast): 3005(w), 2925, 2850, 1745, 1465, 1163 cm^{-1}; ^{1}H nmr($CDCl_3$): δ0.89(m, 3x CH_3), 1.3(m, 3x -($\underline{CH_2}$)$_n$-CH_2CH_2CO-), 1.6(m, 3x -$\underline{CH_2}CH_2CO$-), 2.04(m, -$\underline{CH_2}CH$=CH- of 55), 2.32(t, J=7.5 Hz, 3x -$\underline{CH_2}CO$-), 2.79(t, J=6 Hz, -CH=CH$\underline{CH_2}$CH=CH- of 55), 4.15 and 4.32(4H, 2x dd, J=12, 4.5 and 12, 6 Hz, 2x H_a and 2x H_b), 5.23-5.48(m, H_x and -CH=CH- of 55).

From several fractions of the above flash chromatography of the NFRC-631 mycelial extract, a mixture of fatty acids crystallised upon concentration, mp 59-61°C; ftir($CHCl_3$ cast): 3300-2400(b), 2915, 2850, 1697 cm^{-1}; cims: m/z 386(M+18 for lignoceric acid(24 carbons)), 358(M+18 for behenic acid(22 carbons)), 330(M+18 for arachic acid(20

carbons)), 302(M+18 for stearic acid(18 carbons)); ^{1}H nmr($CDCl_3$): δ0.88(3H, t, J=7 Hz, $\underline{CH_3}(CH_2)_n$-), 1.26(s, $CH_3(\underline{CH_2})_n CH_2CH_2CO$-), 1.63(2H, quintet, J=7.5 Hz, $-\underline{CH_2}CH_2CO$-), 2.35(2H, t, J=7.5 Hz, $-CH_2CO$).

Hydrolysis of triglycerides

A solution of triglycerides (158 mg) in tetrahydrofuran (4 mL) and 10%(w/v) potassium hydroxide (2 mL) was magnetically stirred overnight at room temperature. After evaporation of the tetrahydrofuran in vacuo, the solution was acidified (5 N hydrochloric acid) and extracted with chloroform (5x 20 mL). The combined chloroform extracts were washed with water (3x 25 mL) and brine (3x 25 mL) and dried over sodium sulphate. Filtration and evaporation afforded crude hydrolysis products (96 mg). Crystallisation from ethyl acetate gave mixed fatty acids (11 mg), mp 55-57°C; cims: m/z 302(M+18 for stearic acid(18 carbons)), 298(M+18 for linoleic acid (55)), 274(M+18 for palmitic acid(16 carbons).

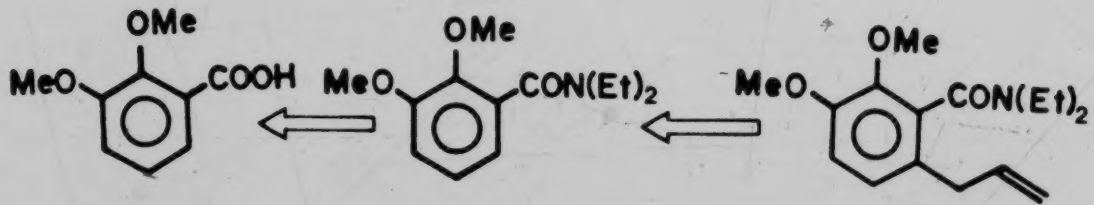
The remainder of the hydrolysis products was subjected to flash chromatography (Skellysolve B-ethyl acetate-acetic acid(gradient), 94:5:1 to 89:10:1 to 84:15:1) to afford additional fatty acids (62 mg), ir($CHCl_3$): 3500-2400(b), 2930, 2860, 1706 cm^{-1}; hreims: m/z calcd. for $C_{18}H_{36}O_2$(stearic acid)(M^+): 284.2715; found 284.2714(8); m/z calcd. for $C_{18}H_{32}O_2$(linoleic acid(55)(M^+): 280.2402; found 280.2400(10); m/z calcd. for $C_{16}H_{32}O_2$(palmitic acid)(M^+):

256.2402; found 256.2403(20); ^{1}H nmr($CDCl_3$): δ0.88(m, $\underline{CH_3}(CH_2)_n$-), 1.26(m, $CH_3(\underline{CH_2})_nCH_2CH_2CO$-), 1.64(m, -$\underline{CH_2}CH_2CO$-), 2.05(m, -$\underline{CH_2}$-CH=CH- of 55), 2.36(t, J=7.5 Hz, -CH_2CO-), 2.79(t, J=6 Hz, -CH=CH$\underline{CH_2}$CH=CH- of 55), 5.35(m, -CH=CH- of 55), 9.7(bs, -COOH).

3. SYNTHETIC STUDIES ON A POTENTIAL IRON-BINDING FUNGAL METABOLITE AND ANALOGUES

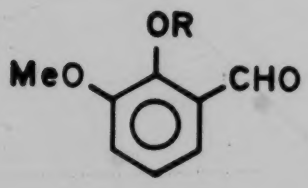
3.1 DISCUSSION AND RESULTS

We envisioned that 12, as well as the natural product analogues 13-15, could be synthesised from the allyl benzamide intermediate 56. Compound 56 should be available by addition of the three-carbon sidechain to benzamide 62, which in turn would be prepared from veratric acid (2,3-dimethoxybenzoic acid, 58). The preparation of 58 from ortho-vanillin (59) has been described by King[66].

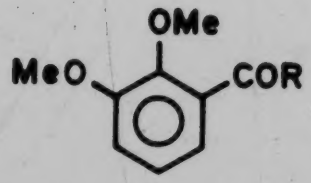


58 57 56

Thus, commercially available 59 was methylated (methyl iodide, potassium carbonate). The yield of 60, reported as



59 R = H
60 R = Me



61 R = Cl
62 R = N(Et)2

80%[66], was improved to >90% by employing a "gentle" reflux

(45°C) and extended reaction times. The aldehyde function of 60 was oxidised (potassium permanganate, potassium bicarbonate) to the corresponding carboxylic acid 58 (97% yield)[67].

As mentioned above, 2,3-dihydroxybenzoic acid (4) has been isolated as a metabolite of the Ceratocystis fungal complex. It is therefore required for iron-binding studies and bioassays. Thus 4 was prepared from 58 by refluxing with hydrobromic acid[66] or by treatment with boron tribromide at room temperature followed by aqueous hydrolysis of the borate ester.

Benzoic acid 58 was converted to diethyl amide 62 via the corresponding acid chloride 61. For the transformation of 58 to 61, distilled oxalyl chloride and distilled benzene were used. A large excess (14 equivalents) of oxalyl chloride was added to a solution of 58 in benzene. After refluxing for one hour the reaction mixture was evaporated in vacuo. This initial attempt afforded only starting material 58. A subsequent effort employed dry benzene (vide infra) and 58 was oven-dried at 100°C for several hours before use. Following treatment with oxalyl chloride (16 equivalents) under reflux, evaporation to dryness yielded a white, crystalline material (61, mp 45-47°C) which was stored in vacuo. The infrared (ir) spectrum (Nujol mull) of this compound shows a carbonyl stretch typical of an acid chloride (1779 cm^{-1}), with no indication of the benzoic acid in the carbonyl or OH regions. However this compound is

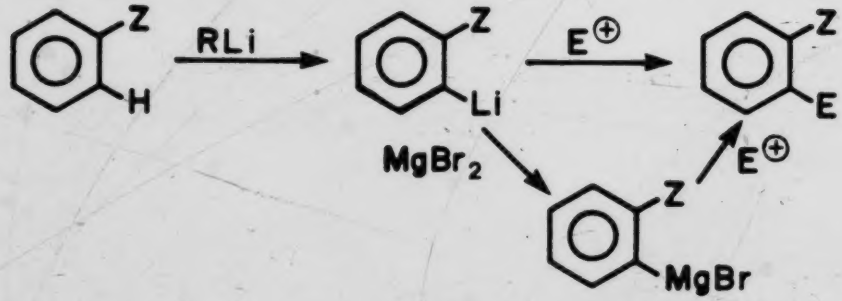
quite susceptible to hydrolysis, since a sample submitted for mass spectroscopy (ms) gives a peak of only 3.5% intensity for $C_9H_9O_3{}^{35}Cl$, while the base peak corresponds to the acid 58. After several days on the vacuum pump the crystalline substance was reexamined. The melting point had risen to 60-63°C and the mass spectrum (ms) gave a peak at m/z 346 (14% intensity), assigned to the anhydride resulting from condensation of the acid 58 with the acid chloride 61.

In order to prepare the benzamide 62, compound 58 (0.66 g) was treated in the manner described above to give 61, which was combined immediately with diethylamine (8 equivalents) in dry benzene and refluxed for one hour. Compound 62 was obtained in 95% yield after flash chromatography. A small amount (2%) of starting material was also isolated. When the reaction was performed on a synthetically practical scale (34 g of 58), crude 62 obtained was purified by flash chromatography and bulb-to-bulb distillation.

The three-carbon side chain was to be appended to 62 *via* a regioselective aromatic substitution. The selectivity accrues from the fact that a wide variety of functional groups (Z) are capable of directing *ortho* metalation of

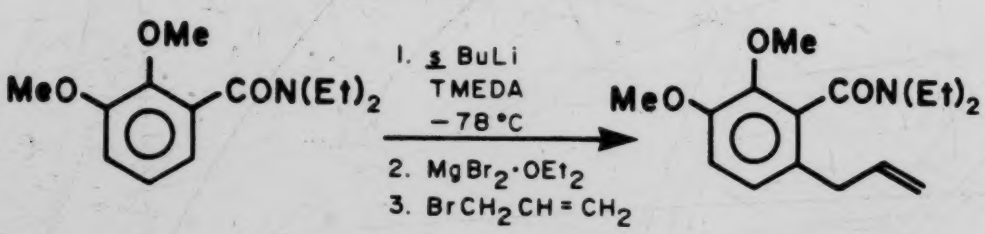
aromatic compounds (Scheme 8)[68]. Both methoxy and

Scheme 8 ortho metalation



N,N-diethylamido substituents are present in 62, and

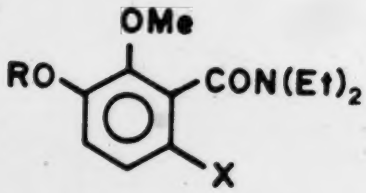
Scheme 9 ortho metalation of 62



62 56

although the former was the first group to be employed in the development of this methodology[69], the latter is the more powerful ortho directing group[70]. The procedure used was that of Snieckus (vide supra)[31]. However initial attempts (Scheme 9) failed to generate any detectable amount of allyl benzamide 56. Instead, by-products 63, 64, and 65 were isolated (<5% yield) in addition to recovered starting

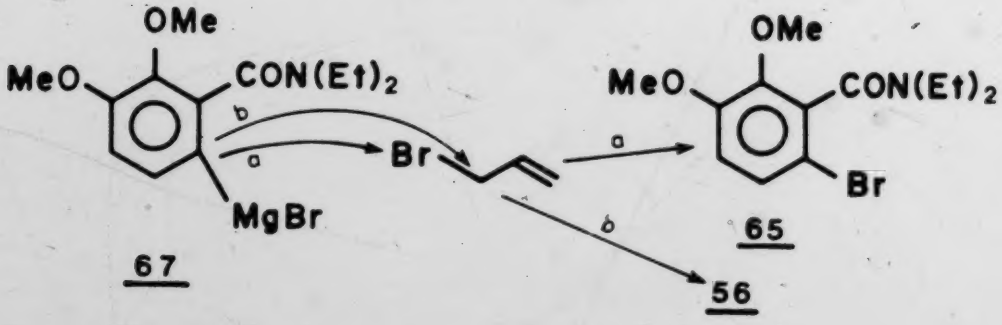
material (>90%). Phenol 64 likely resulted from oxidation



63	R = H	X = H	66	R = Me	X = Li
64	R = Me	X = OH	67	R = Me	X = MgBr
65	R = Me	X = Br			

of the metalated species 67, suggesting incursion of atmospheric oxygen. Vinyl anions are known to give vinyl bromides by reaction with 1,2-dibromoethane or allyl bromide[71]. Therefore it appears likely that bromide 65 forms by reaction with allyl bromide in the undesired mode (Scheme 10, pathway "a").

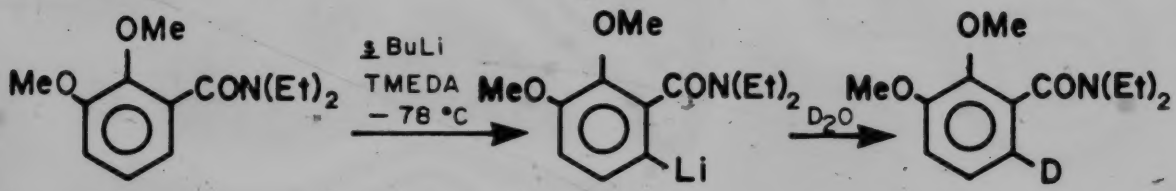
Scheme 10 Formation of bromide 65



Since none of the alkylated product 56 was obtained, it was undertaken to prove the existence of the anions (66 and 67) at various stages of the reaction procedure by deuterium

incorporation. Thus the reaction was quenched with deuterium oxide (D_2O) after addition of s-butyllithium and tetramethylethylenediamine (TMEDA). After several unsuccessful efforts, deuterium incorporation (as determined by ^1H nuclear magnetic resonance spectroscopy (nmr) of 68) was achieved using 10 equivalents each of the base and TMEDA (Scheme 11). None of undeuterated 62 was detected. Complete deuterium

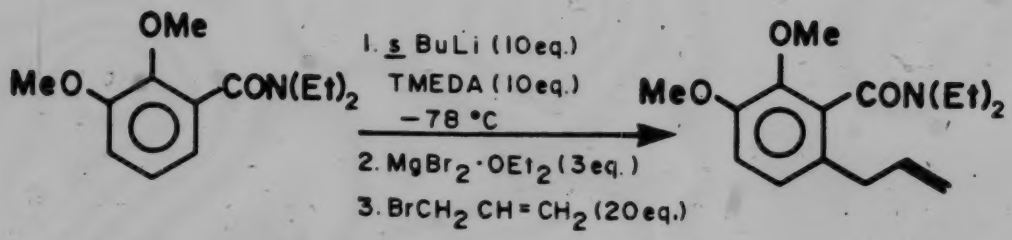
Scheme 11 Quenching of anion 66 with D_2O



62 66 68

incorporation was also observed by adding freshly prepared [72] magnesium bromide etherate after the s-butyllithium/TMEDA, then quenching with D_2O.

Scheme 12 Formation of allyl benzamide 56

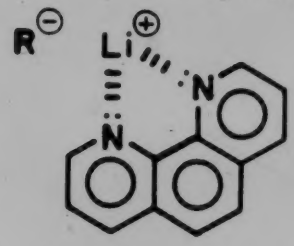


62 56

With the formation of the anion having been demonstrated, attention was returned to the alkylation

reaction. Using the amounts indicated (Scheme 12), 62 (0.108 g) was alkylated in 82% yield. A small quantity (8%) of starting material 62 was recovered. For larger scale preparations of 56, 10 equivalents of s-butyllithium would represent an inconveniently large volume, thus in two subsequent runs, 1,10-phenanthroline was employed to indicate the presence of excess alkyllithium (Scheme 13). However, none of 56 was formed.

Scheme 13 1,10-phenanthroline complex with akyllithium



bright red complex

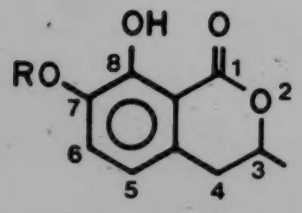
Table 2 Formation of 56 (amounts, yields)

	Equivalents of reagents					Yield(%)	
Entry	62(g)	sBuLi	TMEDA	$MgBr_2 \cdot OEt_2$	$BrCH_2CH{=}CH_2$	56	62
1	0.108	10	10	3	20	82	8
2	0.106	10	10	3	20	12	*
3	0.253	10	10	3	20	33	21
4	0.903	2.5	2.5	3 (solid)	10	30	30
5	0.944	2.5	2.5	3	10	61	13
6	0.894	1.5	1.5	3	10	11	65
7	1.253	2.5	2.5	3	10	68	10
8	1.536	2.0	2.0	3	10	75	10
9	3.038	1.5	1.5	3	3	92	*

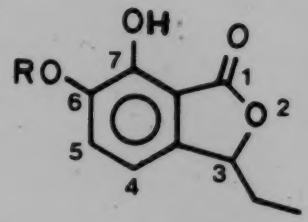
* not isolated

Two repetitions of the

procedure without the phenanthroline indicator afforded 56 in only 12% and 33% yield. Despite these low yields, subsequent runs were performed on "gram" scale since the starting material could easily be re-isolated when the reaction was unsuccessful. These reactions are summarised in Table 2. In entry 4 solid, commercially available magnesium bromide etherate was utilised.



12 R = H
69 R = Me



70 R = H
71 R = Me

With 56 in hand, hydrolysis and cyclisation to the lactone 12 was attempted. A 40 mg sample of 56 in 6 N hydrochloric acid was refluxed for 48 hours. The reaction gave four crystalline products, 12, 69, 70, and 71. The phthalide structure is presumably formed by isomerisation of the carbon-carbon double bond prior to cyclisation. Following flash chromatography, compounds 12 and 69 were obtained as a 5:1 mixture (16 mg, 55%) and 70 and 71 as a 1:1 mixture (4 mg, 20%). That 69 is 7-methoxymellein was evidenced in the ^{1}H nmr spectrum of 12 + 69 by broad singlets at δ11.05 and δ11.24 (hydrogen-bonded phenolic protons) and a singlet at δ3.89 (3H,

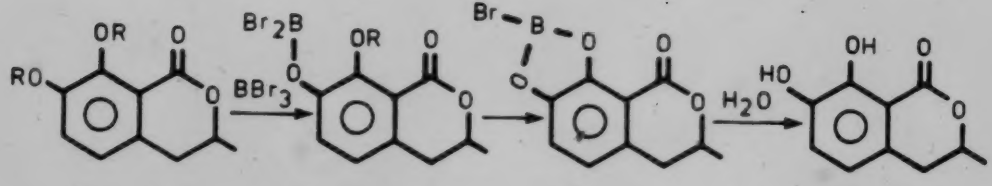
OCH_3).

The structures of 70 and 71 were indicated by ^1H nmr of the mixture. A signal appearing at δ5.45 (dd) is assigned to the proton at C-3. The 3-ethyl substituent is suggested by a complex signal centred at δ2.0 for the methylene group and a triplet at δ1.00 corresponding to the methyl group.

In order to demethylate at the 7-position in 69, the mixture of 12 and 69 was subjected to treatment with boron tribromide (BBr_3). Following the reaction, only 12 was present (by tlc), and the ^1H nmr characteristics of this material are in excellent agreement with those published for the natural product[26].

Although one equivalent of BBr_3 is normally required per site of potential reaction (i.e. ArOR), in the case of catechols only one mole BBr_3 is needed per mole of substrate, as shown in Scheme 14.

Scheme 14 **Reaction of BBr_3 with a catechol**



Subsequently, the product mixture obtained from the reaction of 56 with 6 N hydrochloric acid was not chromatographed but simply treated with BBr_3 to afford 12 and 70, which were then separated by flash chromatography. A longer reaction time (96 hours) with 6 N hydrochloric acid

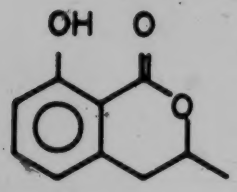
(Table 3, entry 2) did not give complete demethylation (69

Table 3 Formation of 12 and 70 (amounts, yields)

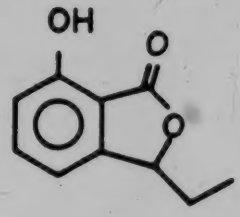
Entry	56 (g)	Reaction time with 6 N HCl (hr.)	Yield (%) 12	70
1	0.040	48	55	20
2	0.092	96	64	22
3	0.122	48	55	20
4	0.457	48	62	13
5	0.686	48	56	*
6	1.236	48	62	17

* not isolated

was still evident by tlc), and a comparison of entries 1 and 2 shows that the reaction time with hydrochloric acid does not have much effect on the overall relative yields of 12 and 70.



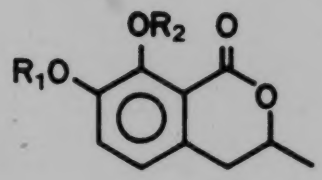
19



72

Isoochracin (72) is a natural product isolated from the same fungus (Hypoxylon howieanum[73]) as mellein (19, also known as ochracin). Thus compound 70 can be named 6-hydroxyisoochracin. Since 6-hydroxyisoochracin possesses the ortho-acyl-substituted catechol required for siderophore-like behaviour, it will be tested in the planned

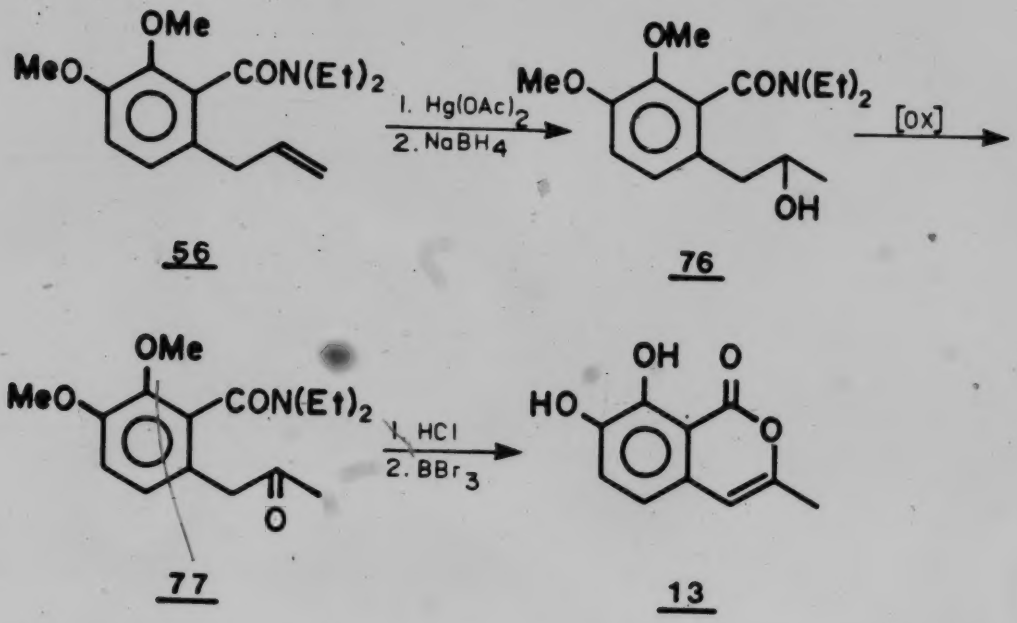
iron-binding studies and bioassays (vide supra).



12 $R_1 = R_2 = H$ 74 $R_1 = H$ $R_2 = Me$
73 $R_1 = Me$ $R_2 = H$ 75 $R_1 = R_2 = Me$

With compound 12 in hand, we turned our attention to the preparation of 13- 15. Introduction of a carbon-carbon double bond into 12 to give 13 was considered. A literature procedure[27] indicates that benzylic bromination could be performed on the dimethyl derivative (75) of 12.

Scheme 15 Synthetic route, 56 to 13



Dehydrohalogenation of the brominated compound, followed by

deprotection of the phenols would then give rise to 13. Thus methylation (methyl iodide, potassium carbonate) of 12 was attempted. However, despite heating for 22 hours in the presence of a large excess of alkylating reagent, the reaction did not proceed to completion. After flash chromatography, ^{1}H nmr spectra of the products showed the presence of starting material 12, the 7-methoxy derivative 73, the 8-methoxy derivative 74, and the 7,8-dimethoxy compound 75, in a ratio of 2:4:2:1. Since the formation of 75 was not straightforward (however, see 142 and 144 below) a different approach to 13 was pursued. This proposed synthetic route from 56 to isocoumarin 13 is shown in Scheme 15.

The oxymercuration-demercuration of 56 was carried out in order to hydrate the olefin in the Markovnikov sense. The initial effort was performed on 18 mg of 56, and gave, after flash chromatography of the crude products, a small amount (1 mg, 6%) of starting material (R_f 0.7), a mixture of two relatively polar (R_f 0.29 and 0.22) compounds (14 mg, 73%), and a polar (R_f 0.05) component (*vide infra*). The ^{1}H nmr spectrum (Figure 8) of the mixture of relatively polar compounds showed that each of the two structures possess two

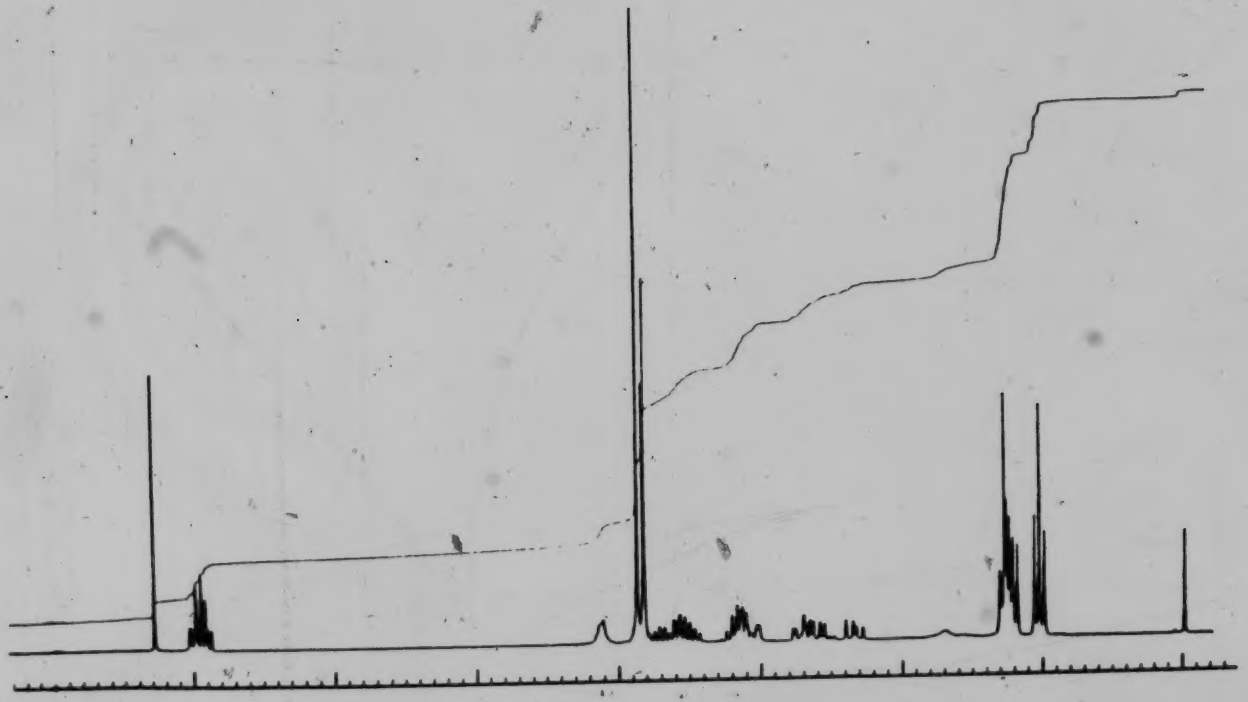
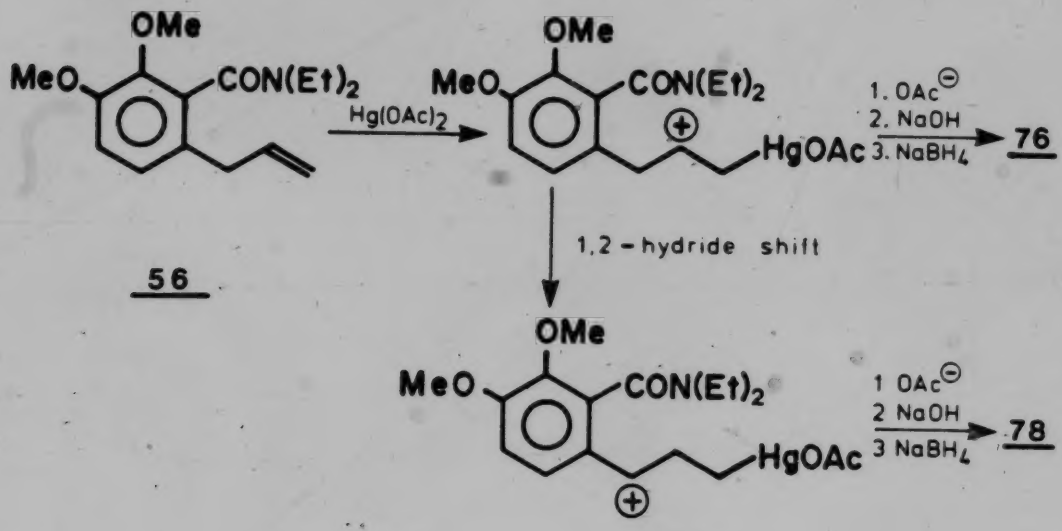


Figure 8. 200 MHz ^{1}H nmr spectrum ($CDCl_3$) of alcohol 76

methoxyl groups and the 3° amide functionality.

Scheme 16 Possible formation of alcohols 76 and 78



signal at δ4.1 was changed to a series of sharp lines by addition of D_2O. Broadened signals at δ1.71 and δ3.06 disappeared upon D_2O exchange. It was apparent that a mixture of alcohols had been formed. Since a 1,2-hydride shift of the initially formed 2° carbonium ion would give rise to a more stable benzylic cation, it was thought that the mixture might consist of isomeric alcohols 76 and 78 (Scheme 16). In order to acquire more information on the structure of the two alcohols, they were treated with pyridine and acetic anhydride at room temperature to form the corresponding acetates. In the ^1H nmr spectrum (Figure 9) of the products (which gave a single spot on tlc), the acetyl methyl groups appear as singlets at δ2.01 and δ2.02.

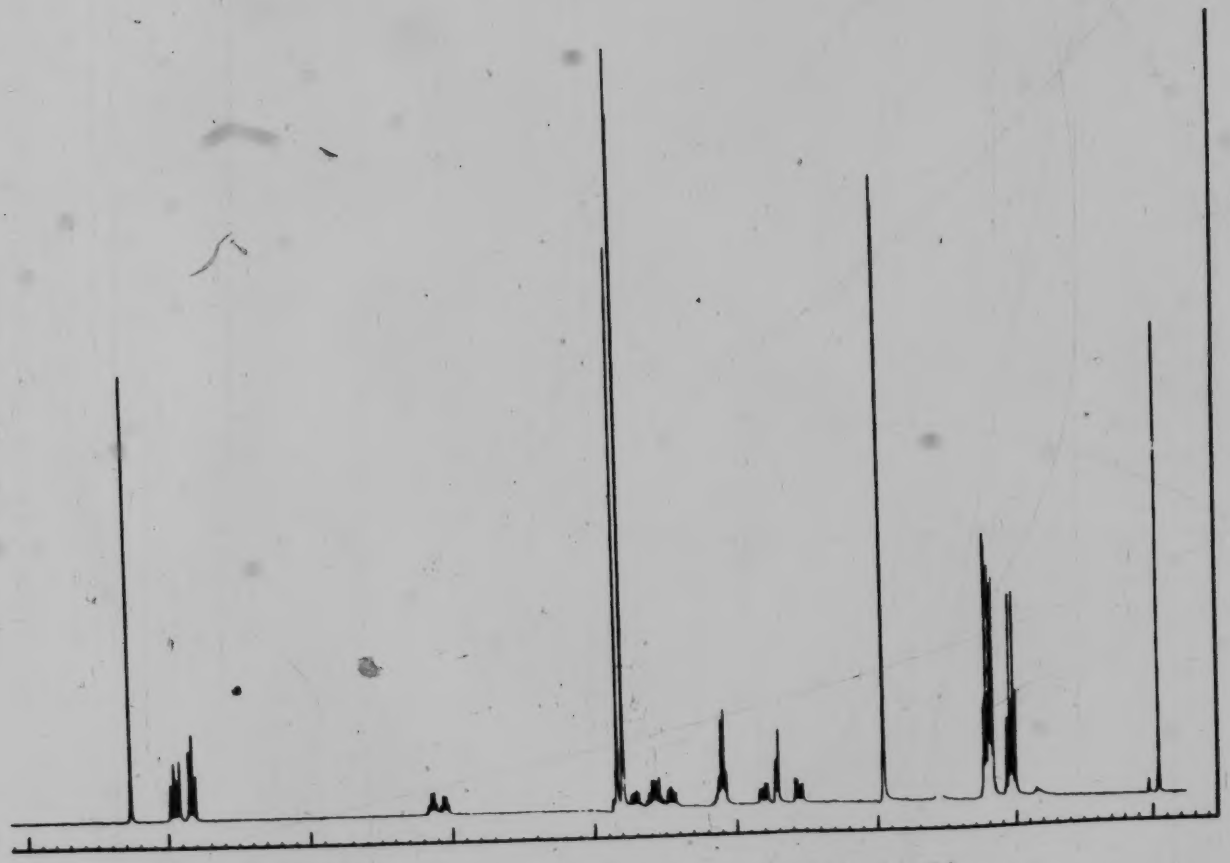
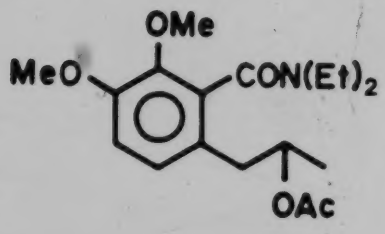
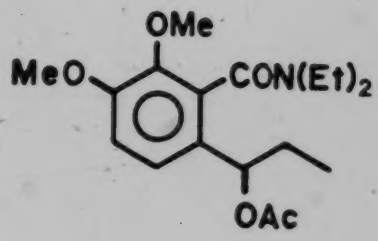


Figure 9. 400 MHz ^{1}H nmr spectrum ($CDCl_3$) of acetate 79

As anticipated, the signal for the carbinyl proton in the alcohol mixture was shifted downfield[74]. Thus the δ4.1 signal in the alcohol mixture was replaced by signals at δ5.09 and δ5.17 (each a sextet, J=6.5 Hz). Assuming a 1:1 ratio of the two compounds, these two signals together integrate for two protons. The carbinyl proton in acetate 79 could appear as a sextet, but that of acetate of 80 should appear as a triplet. Since two sextets are observed,



79



80

the conclusion was drawn that the two acetates are rotational isomers, and therefore the two parent alcohols are rotational isomers of 76. Examination of molecular models shows that the bulky 3° amido and 2-propanolic aromatic substituents experience serious steric interactions with each other, thus it seems conceivable that two distinct conformations (observable by tlc and ^{1}H nmr) exist. Also in keeping with this proposal are signals in the ^{1}H nmr spectrum of the acetates for the benzylic methylene protons (δ2.60 (1H, dd, J=14.5, 6.5 Hz) and δ2.85 (1H, dd, J=14.5, 6.5 Hz) for one isomer; δ2.76 (2H, d, J=6.5 Hz) for the other isomer) and for the methyl group of the three-carbon

sidechain (δ1.325 (3H, d, J=6.5 Hz); δ1.335 d, J=6.5 Hz). The ^{1}H nmr spectrum of the parent alcohols can be seen as consistent with the idea of rotational isomers since four benzylic protons are observed (each giving a dd) and two methyl doublets are discernible at δ1.20 (J=6 Hz) and δ1.26 (J=6 Hz). The chemical ionisation mass spectrum (cims) of the alcohol mixture gives a single large peak at m/z 296 (M+1, 100%).

An attempt to separate the two isomers of 76 by preparative tlc (chloroform-ethyl acetate, 50:50, three elutions) was unsuccessful.

Table 4 Oxymercuration-demercuration (amounts, yields)

Entry	56 (mg)	$Hg(OAc)_2$ (eq.)	Reaction time (hr.)	# products (tlc.)	Yield (%) 76	56
1	18	1.0	0.5	2	73	6
2	23	1.2	21	3	*	*
3	25	1.2	21	3	*	*
4	14	1.1	91	4	*	*
5	46	1.0	0.17	2	17	80
6	82	1.1	0.5	2	62	17
7	125	1.1	1.0	2	65	15
8	49	1.1	2.0	3	50	4
9	57	5.0	0.5	3	57	9

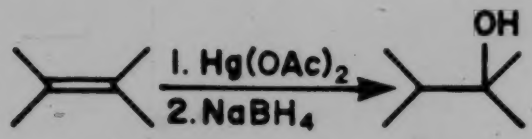
* not isolated

A variable temperature ^{1}H nmr experiment was conducted to try to cause the two sets of signals of the rotational isomers to coalesce into one set. The ^{1}H nmr of 76 (in toluene-d_8) was recorded at 200 MHz at ambient temperature,

50°C, 75°C, and 100°C. However, only slight line broadening was observed. Temperatures above 100°C were not attainable with the high field nmr variable temperature equipment available. An attempt was made to do the experiment at 80 MHz, but the resolution at this field strength is not sufficient to observe the two isomers at ambient temperature.

In subsequent oxymercuration-demercuration reactions of 56, it was observed that the reaction time with mercuric acetate (before addition of sodium hydroxide and sodium borohydride) has a profound effect on the yield of the desired alcohol 76, as well as on the number of reaction products. As shown in Table 4, starting material 56 was always isolated, regardless of the reaction time. However, prolonged times (entries 2-4) resulted in the appearance of additional products. It has been shown (Scheme 17)[75] that long reaction times can be deleterious to the success of the

Scheme 17 Effect of reaction time with $Hg(OAc)_2$



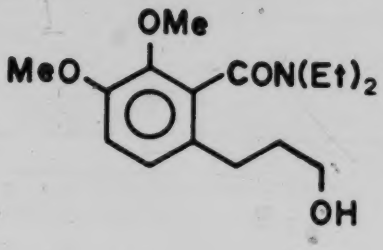
81 82

reaction time	yield (%)
35 min.	86
2.25 hr.	44

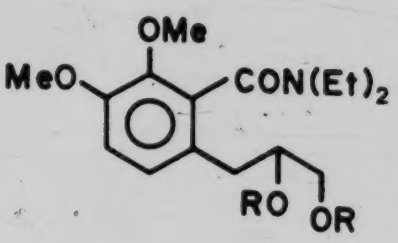
reaction. Reaction times of 0.5 or 1.0 hours (entries 1,6,7) gave the best yields of 76 and only one by-product (more polar than 76, *vide infra*). A yield of only 17% of 76 (entry 5) was obtained with a shorter reaction time. The

yield of 76 was slightly less than optimum, and two by-products were isolated (one of these is the above-mentioned polar by-product) with a reaction time of 2.0 hours (entry 8) and by using excess mercuric acetate (entry 9). These two by-products were isolated from the reaction mixture of experiment 8. Their structures were elucidated as alcohol 83 and diol 84. Like 76, 83 gives two sets of signals in the ^{1}H nmr spectrum, and therefore presumably exists as two rotational isomers. However, unlike 76, only a single spot is observed for 83 by tlc. The structure of 83 is apparent from the following data. Four protons (each a dd) appear between δ1.75 and δ2.22 (^{1}H nmr) which can be assigned to the homobenzylic methylene group of the two isomers of 83. No signals are present in this region of the ^{1}H nmr spectrum of 76. In addition, the two doublets corresponding to the sidechain terminal methyl groups in 76 are absent from the methyl region in the ^{1}H nmr spectrum of 83. Otherwise the ^{1}H nmr spectra of 76 and 83 are similar, consistent with the remainder of structure 83. By hreims, 83 displays the same major fragmentation peaks as 76 (i.e. m/z 251, 236, 220, 205, 179) but 83 shows a more intense signal for the loss of H_2O (m/z 277, 18%) than 76 (m/z 277, 0.1%). The cims of 83 gives an M+1 peak (m/z 296, 54%) and a base peak for M+1-H_2O (m/z 278). The hydroxyl

and amide carbonyl absorptions appear at 3600-3100 and 1612 cm^{-1} respectively (ir).



83

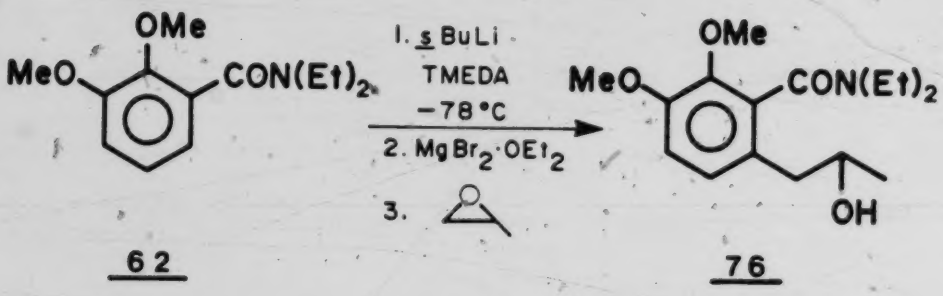


84 R = H
85 R = $COCH_3$

The structure of diol 84 (which also exists as two rotational isomers but appears as one spot by tlc) is consistent with the following spectral data. The ^1H nmr spectrum exhibits benzylic protons (each a dd) between δ2.44 and δ2.88. No signals are present in the region δ1.8-2.4 (where the homobenzylic protons of 83 appear) and the doublets arising from the methyl groups of the sidechain in 76 are missing in the spectrum of 84. The remainder of the ^1H nmr spectrum of 84 is in agreement with the proposed structure. The hreims does not give a parent peak but does show the major fragmentation peaks common to 76 and 83 at m/z 251, 236, 220, and 179. An M+1 (m/z 312, 100%) peak is present in the cims of 84. The ir spectrum displays OH (3600-3100 cm^{-1}) and amide carbonyl (1605 cm^{-1}) absorptions. The diacetate 85 was prepared by overnight treatment 84 with pyridine/acetic anhydride at room temperature. The ^1H nmr spectrum exhibits six protons (three for each isomer)

between δ3.97 and δ5.41 due to carbinylic protons. Four acetyl methyls (two for each isomer) are evident at δ2.02, 2.03, 2.07, and 2.08. The "methylene- 2° acetate - 1° acetate" sequence in the three-carbon sidechain was verified by decoupling experiments. The hreims of the diacetate 85 gives the parent peak (m/z 395, 24%) plus consecutive losses of acetic acid (m/z 335, 100%) and acetyl radical (m/z 276, 81%). The ir spectrum shows carbonyl stretches for the acetates (1742 cm^{-1}) and amide (1630 cm^{-1}).

Scheme 18 Formation of alcohol 76 by ortho metalation



62 76

A more direct approach to 76 from 62 involves the use of propylene oxide instead of allyl bromide as the electrophile in the ortho-metalation reaction (Scheme 18). A similar reaction was employed in a synthesis of mellein (19, vide supra)[27]. The best yields of 76 were 66-73%, as

shown in Table 5. By-products were formed which appear at

Table 5 Formation of 76 (amounts, yields)

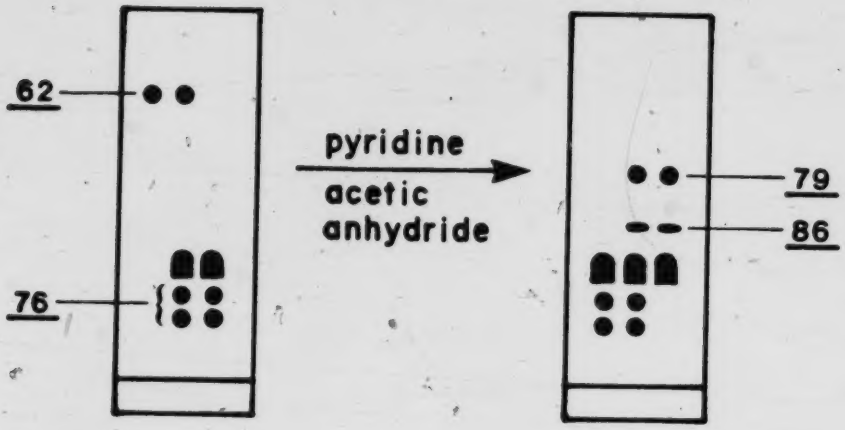
Equivalents of reagents

Entry	62 (g)	s BuLi	TMEDA	$MgBr_2 \cdot OEt_2$		Yield (%) 76	62
1	0.991	2.5	2.5	3	10	11	60
2	1.154	2.5	2.5	3	10	47	52
3	0.346	2.5	2.5	3	10	5	60
4	0.968	2.5	2.5	3	10	7	65
5	1.206	2.5	2.5	3	10	66	15
6	1.222	2.5	2.5	0	10	24	28
7	1.054	1.2	1.2	3	5	44	30
8	1.426	2.0	2.0	3	10	66	18
9	3.269	1.5	1.5	3	3	73	7

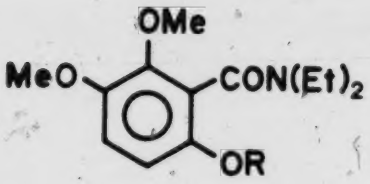
slightly higher R_f on tlc than the desired alcohol 76. Unreacted starting material 62 was separated from the reaction products by flash chromatography. It was found that 76 could best be further purified by subjecting the mixture of reaction products to acetylation conditions (Scheme 19), then purifying acetate 79 by flash chromatography, and deacetylating (potassium carbonate, methanol, room temperature, quantitative yield) to afford 76. The only by-product of the ortho-metalation reaction which was acetylated is phenol 64. Its acetate 86 (Scheme 19) was isolated (44 mg, 2% from 62) following acetylation of the combined crude reaction products from entries 1-2, Table 5. Hydrolysis of the crystalline 86 afforded 64.

Acetate 86

Scheme 19 tlc: alcohol 76 to acetate 79

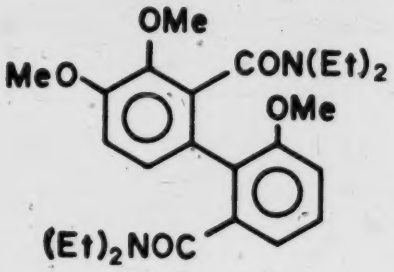


was also prepared by acetylation of 64.



64 R = H

86 R = $COCH_3$



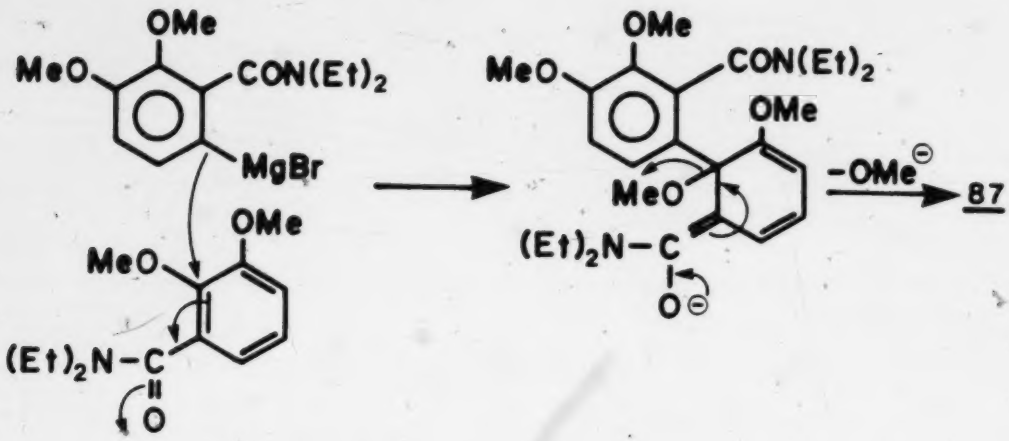
87

Of the by-products (entries 1-2) which did not acetylate, one eluted in quite pure form from the flash chromatography of the acetylation products. Further purification by preparative tlc and flash chromatography gave compound 87 (9 mg, 0.2% from 62). Its tentatively assigned structure is based on its ^1H nmr, ms, and ftir data. The ^1H nmr spectrum shows five aromatic protons, two

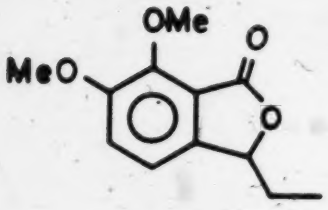
of which are ortho-coupled (2x d, J=8 Hz). The other three are contained in a contiguously trisubstituted benzene nucleus (1H, dd, J=7.5 Hz(ortho); 1H, dd, J=7.5, 1 Hz(ortho and meta); 1H, dd, J=7.5, 1 Hz(ortho and meta). Three methoxy groups are evident, as well as the multiplets of the methylenes of the amide functionalities. That two amides are present is shown by the four triplets of the amide methyls. An amide absorption appears at 1628 cm^{-1} in the ir spectrum. The hreims gives the main indication of the dimeric nature of the structure (m/z 44, 36%), and this is supported by cims, which shows an M+1 peak at m/z 443 (100%). The mechanism in Scheme 20 could explain the formation of 87. The proposed mechanism is supported by the fact that some Grignard reagents are known to add 1,4 to aromatic systems[76]. Later alkylations of 62 using propylene oxide (entries 3-9) also produce non-acetylating by-products (which were not investigated (with the exception of entry 6)) but did not give phenol 64 (however, see (R)-alcohol 128

below)..

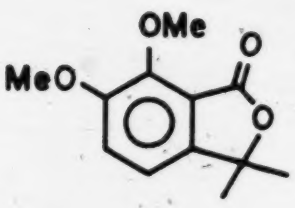
Scheme 20 Formation of biphenyl 87



Following hydrolysis of acetate 79 to the alcohol 76



88

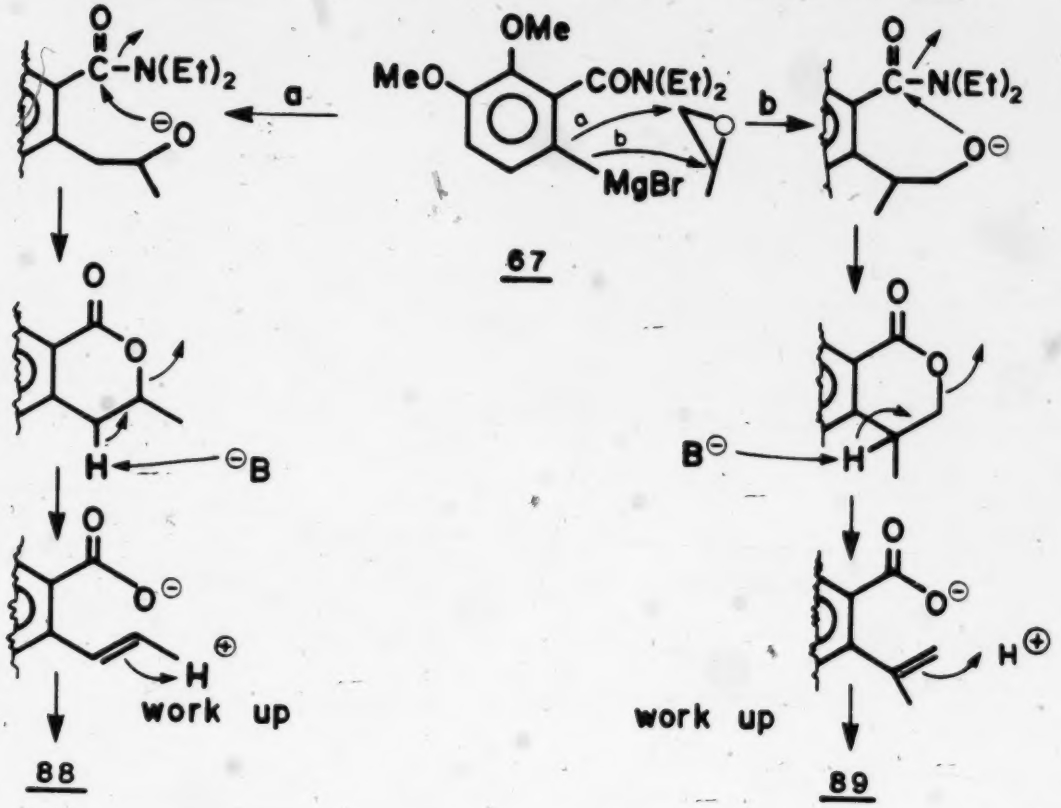


89

(vide supra), small amounts of non-polar materials were consistently observed by tlc. These were isolated (<4% from 62) by flash chromatography (which also served to purify 76 for the next synthetic step) and identified as isomeric phthalides 88 and 89. These compounds went undetected prior to the acetylation reaction, since their structures suggest that they are by-products of the ortho-metalation reaction of 62. The formation of 88 and 89 is rationalised in terms

of the mechanism in Scheme 21.

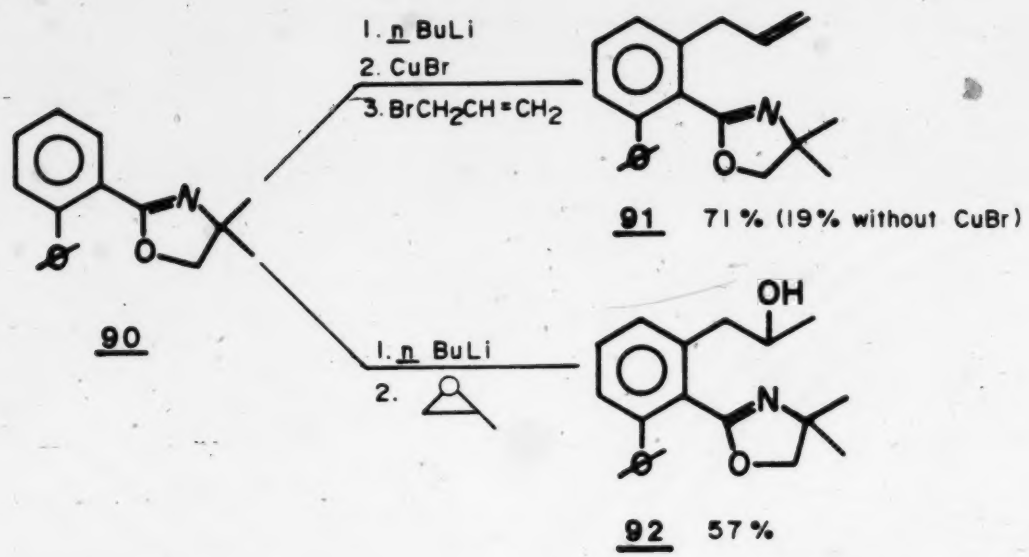
Scheme 21 Formation of phthalides **88** and **89**



The ortho metalation reaction listed under entry 6 was carried out (in the absence of magnesium bromide etherate) following the discovery of the results of Ellefson[77] shown

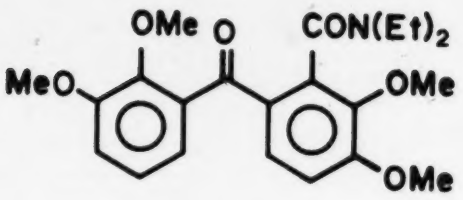
in Scheme 22. It should be noted that in Scheme 22 the

Scheme 22 Ellefson's ortho metalation reactions

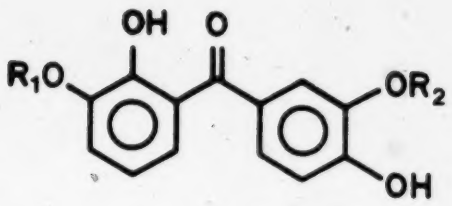


reaction with propylene oxide is performed without formation of the cuprate. The reaction in entry 6 gave, in addition to 76 (24%), a substantial amount (11%) of benzophenone 93. Purification of 93 was facilitated by acetylation, which made 93 easily separable by flash chromatography from acetate 79. The ^{1}H nmr spectrum of 93 shows the structure is composed of two benzene nuclei, one contiguously tetrasubstituted (2x 1H, 2x d, J=8 Hz(ortho)) and the other contiguously trisubstituted (1H, dd, J=7.5 Hz(ortho); 1H, dd, J=7.5, 1.5 Hz(ortho and meta); 1H, dd, J=7.5, 1.5

Hz(ortho and meta)). There are four methoxy substituents



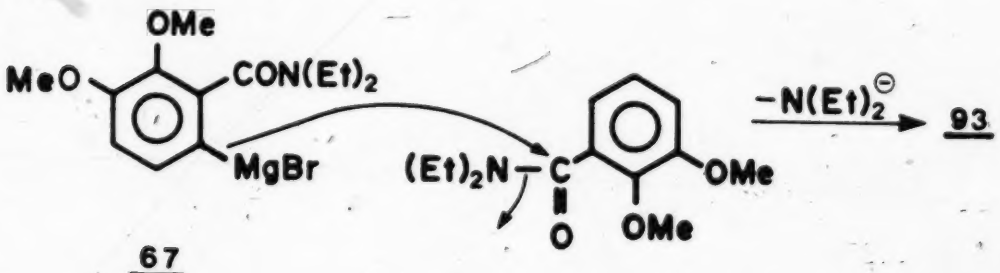
93



94 R_1 = H R_2 = Me
95 R_1 = Me R_2 = H
96 R_1 = R_2 = H

(δ3.73, 3.88, 3.89, and 3.91) but only one amide, as evidenced by the presence of two methyl triplets (δ1.07 and 1.15). The ir spectrum shows the amide carbonyl at 1636 cm^{-1}. A benzophenone is indicated by a carbonyl absorption at 1665 cm^{-1}, as well as a resonance at δ193.7 (^{13}C nmr). The structure of 93 is also consistent with the hreims (m/z 401, 7%). Its formation is explained in terms of the mechanism in Scheme 23. Benzophenones of this nature also have been obtained by Snieckus[31] from his ortho metalation procedure.

Scheme 23 Formation of benzophenone 93



67

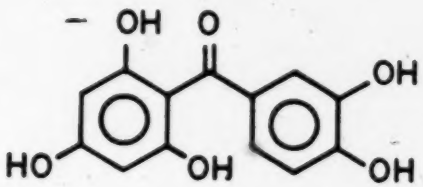
Benzophenone 93 was subjected to acid hydrolysis conditions (6 N hydrochloric acid, reflux, 48 hours). The crude products were treated with boron tribromide followed by aqueous hydrolysis of the borate esters to give a mixture of phenolic compounds. Three products were separated by flash chromatography. Examination of the products by ^{1}H nmr revealed that demethylation was incomplete, since two of the compounds, 94 and 95 (R_f 0.57 and 0.41 respectively), are monomethoxy derivatives of the third, most polar (R_f 0.33) component (96). Structure 96 is easily assigned based on the known structure of compound 93 and the fact that 96 contains no methoxy groups. Assignment of the ^{1}H nmr spectrum of 96 aids in establishing the structures of 94 and 95. Thus the signal at δ7.29 (1H, d, J=2.2 Hz) must represent the H-2' proton since it has only a meta coupling. A coupling constant (2.2 Hz) common to δ7.29 and δ7.18 places the proton of the latter signal at H-6'. The ortho coupling constant (8.2 Hz) of the δ7.18 peak requires that H-5' gives rise to the signal at δ6.95 (1H, d, J=8.2 Hz). The resonance at δ6.81 (1H, dd, J=7.75, 7.75 Hz) is assigned to H-5 since this proton is situated ortho to H-4 and H-6. The remaining signals at δ7.09 (1H, dd, J=7.75, 1.5 Hz) and δ7.16 (1H, dd, J=7.75, 1.5 Hz) must be due H-4 and H-6 although their assignments are interchangeable.

In the ^{1}H nmr spectrum of 94 determined in acetone-d_6+D_2O, the chemical shifts of protons H-4, 5, and 6 (δ7.105, 6.82, 7.15, respectively) are almost identical to

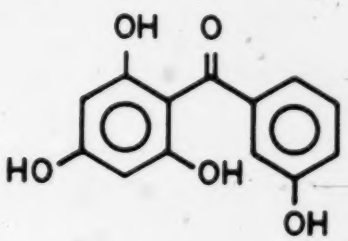
those of the corresponding protons in 96. However, H-5' in 96 is shifted upfield by 0.14 ppm compared to H-5' (δ7.09) in 94. Therefore the methoxy group in 94 is assigned to C-3' based on the smaller meta shielding effect on H-5' in 94 than in 96[78].

Compound 95 gives a ^{1}H nmr spectrum (acetone-d_6+D_2O) spectrum in which H-2', H-5', and H-6' are essentially unshifted from their locations in 96. Thus it appears unlikely that the methoxy substituent in 95 is located at C-3' (94) or C-4'. Since the ^{1}H nmr ($CDCl_3$) of 95 shows a hydrogen-bonded phenolic proton at δ11.9 due to the C-2 OH, the methoxy group of 95 must be assigned to C-3.

Compound 96 has a structure similar to benzophenone natural products maclurin 97[79] and 2,3',4,6-tetrahydroxybenzophenone 98[80].



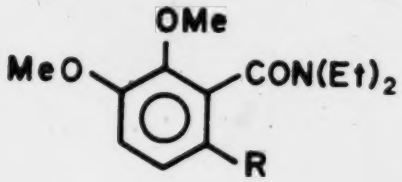
97



98

Alcohol 76 was oxidised to ketone 77 in order to establish the correct level of oxidation in the three-carbon sidechain for the preparation of isocoumarin 13. Three methods of oxidation were investigated. Jones[81] oxidation

resulted in poor yields (41, 44%), while pyridinium chlorochromate[82] gave an improved (74%) yield of 77. However both approaches suffer from the disadvantage that they also produced aldehyde 99 (ca. 3%). Compound 99 is only slightly more polar (R_f 0.34) than ketone 77 (R_f 0.42) and thus makes purification of 77 difficult. Swern[83] oxidation of 76 was most satisfactory since the yields were high (91-94%) and none of 99 was formed. Unlike 76, ketone



77 R = CH_2COCH_3
99 R = CHO

77 does not show pronounced rotational isomerism at room temperature. However, some rotational interdependence between the 2-propanoyl and 3° amido substituents is indicated, since the protons (^{1}H nmr, Figure 10) of the benzylic methylene group appear as doublets (δ3.56 and δ3.70, J=16 Hz).

It was hoped that acid hydrolysis of the 3° amide functionality of 77 would give the keto-carboxy intermediate 100 (Scheme 24), which would presumably cyclise and

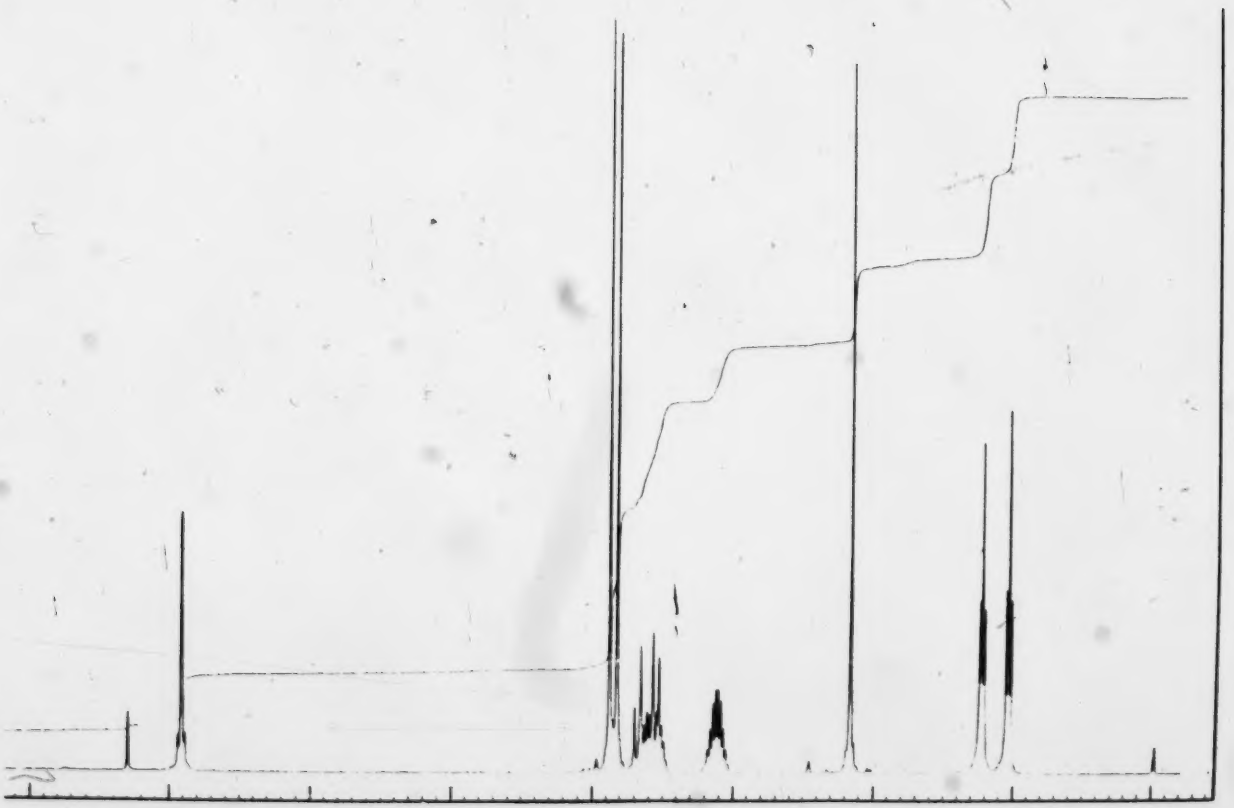
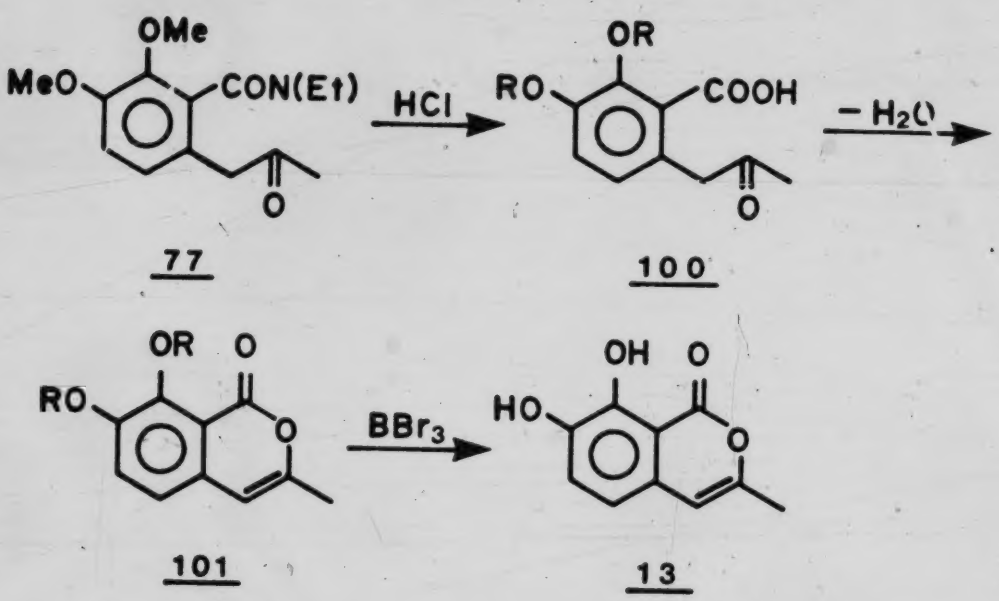


Figure 10. 400 MHz ^{1}H nmr spectrum ($CDCl_3$) of ketone 77

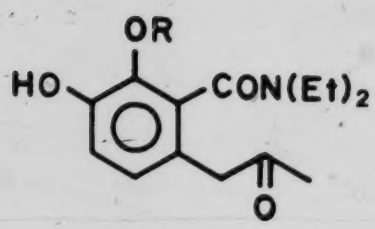
dehydrate under the reaction conditions. Reaction with

Scheme 24 Route, Ketone 77 to isocoumarin 13



boron tribromide to complete the demethylation should then afford 13. Treatment of 77 with 3 N hydrochloric acid (reflux, 24 hours) gave no hydrolysis. Starting material (43%) and phenol 102 (8%) were the only compounds observed in the tlc of the crude products. They were separated by flash chromatography. The structure of 102 was assigned on the basis of its spectral properties. The ^1H nmr spectrum is very similar to that of 77. A single methoxy group appears (δ3.93) along with a singlet at δ5.75 due to a phenolic proton. The ir spectrum shows OH absorption (3600-3040 cm^{-1}) and carbonyls at 1708 and 1625 cm^{-1}. The parent peak for 102 is prominent in its hreims (m/z 279,

60%).



102 R = Me
103 R = H

A shorter reaction time (reflux, 6 hours) with more concentrated hydrochloric acid (6 N) resulted in the isolation of unchanged 77. However longer reaction periods (Table 6) with 6 N hydrochloric acid completely consumed 77.

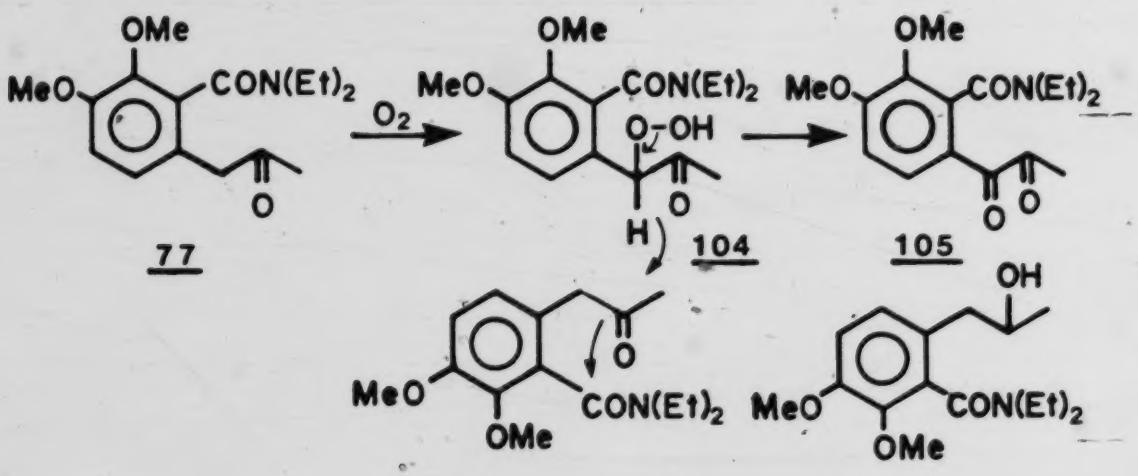
Table 6 Hydrolysis of 77 (amounts, yields)

Entry	77 (g)	Reaction time with 6 N HCl (hr.)	Yield (%) 12+13	Yield (%) 13	Ratio 12:13
1	0.015	20.5	—	—	—
2	0.066	67	7	—	1:2
3	0.056	42	14	—	2:1
4	1.381	168	23	—	1:2.5
5	0.035	144	—	30	—
6	0.244	136	—	24	—

Phenol 102 was the only compound isolated from the crude product of entry 1. Examination of the crude products from entries 2 and 3 by tlc showed more complex mixtures, and indicated the presence of diphenol 103 (vide infra) in

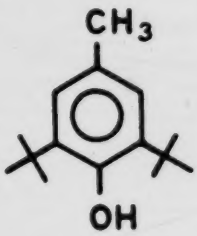
addition to the desired isocoumarin 13. Each of these mixtures was each subjected to treatment with boron tribromide. The products isolated (by flash chromatography) from these reactions were mixtures of 12 and 13 (in very low yields). Since 103 appeared among the crude products of entries 2 and 3, it was thought that a longer reaction time with 6 N hydrochloric acid might give more complete amide hydrolysis. However the crude material isolated from the reaction (entry 4) showed at least six compounds (tlc) besides the least polar

Scheme 25 Auto-oxidation of ~~Ketone~~ 77



mixture of 12 and 13. It was found that if ketone 77 was distilled prior to reaction with 6 N hydrochloric acid, formation of 12 was almost completely suppressed. Thus from entries 5 and 6, compound 13 isolated after the boron tribromide reaction contained little or none of 12 (as determined by ^{1}H nmr). The generation of 12 when

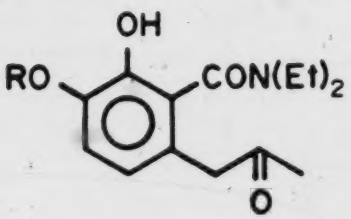
77 is not distilled may be due to formation of hydroperoxide 104 (Scheme 25) by air oxidation at the doubly-activated benzylic methylene group in 77. Collapse of the hydroperoxide with concomitant hydride transfer would lead to the formation of 76 and thus to 12. The oxidation product (105, vide infra) which would result was not detected, however. In order to prevent such auto-oxidation-reduction reactions from occurring, an antioxidant (butylated hydroxytoluene, 106) was added to a reaction mixture containing undistilled ketone 77 and 6 N hydrochloric acid. However, a mixture of 12 and 13 was isolated. The mechanism by which 12 is formed remains unclear.



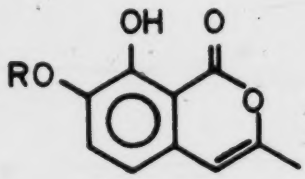
106

An alternate route to isocoumarin 13 from ketone 77 was studied. It was discovered that attempted distillation of ketodiphenol 103 resulted in the formation of 13. Compound 103 was prepared in crude form by treatment of ketone 77 with boron tribromide. Purification of 103 by flash chromatography was possible but in later experiments it was used in impure form in the preparation of 13. A less-polar

(tlc) component present in the crude reaction mixture was presumed to be 7-methoxy-ketophenol 107 (vide infra). It was found that the reaction of 77 with boron tribromide is sensitive to the amount of reagent used. The use of 1.0 equivalent boron tribromide appears (tlc) to maximise the yield of 103 and 107. The cims of this crude reaction product gives only five major peaks: m/z 280, 91% (M+1 for 107); 266, 100% (M+1 for 103); 207, 23% (M+1-$NHEt_2$ for 107); 192, 41% (M+1-$NHEt_2$ for 103); 74, 16% ($NHEt_2$+1). When 1.1-1.5 equivalents of boron tribromide are employed, substantial amounts of 103 and 107 are evident by tlc, but the use of 2.5 equivalents of BBr_3 results in a mixture of products containing very little (tlc) of 103 and 107.



103 R=H
107 R=Me

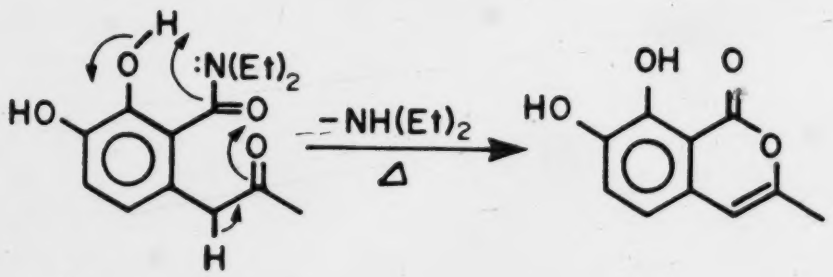


13 R=H
108 R=Me

"Pyrolysis" of the crude mixture of 103 and 107 in a Kugelrohr bulb-to-bulb distillation apparatus (in vacuo) gave "distilled" material containing isocoumarin 13 and 7-methoxyisocoumarin 108. The yields were 12% and 7% respectively over two steps from 77 when the sequence was performed on a small scale (beginning with 63 mg of 77).

The formation of 7-methoxyisocoumarin (108) is indicated by singlets at δ11.20 (hydrogen-bonded phenolic proton) and δ3.95 (methoxy) in the ^{1}H nmr spectrum. The fact that 103 and 107 can be heated to afford the corresponding isocoumarins indicates that a phenolic group ortho to the amide assists in a thermally-induced elimination of diethylamine. Molecular models show that the lone pair electrons on the nitrogen atom of the amide in 103 or 107 closely approach the proton of the ortho phenol in the conformation shown in Scheme 26. The result is a transition state containing two six-membered rings. This hypothesis is supported by the fact that ketone 77 is unchanged upon distillation and that none of 8-methoxyisocoumarin is isolated from the "pyrolysis" products.

Scheme 26 Thermally induced formation of 13

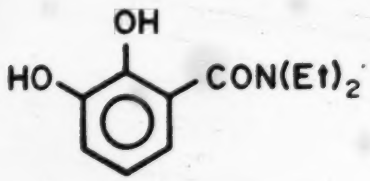


103 **13**

In preparing somewhat larger quantities of 13, the following procedure was employed. A solution of ketone 77 in methylene chloride was treated with 1.0 equivalent of boron tribromide at -78°C and the reaction mixture was allowed to warm to room temperature overnight. Following

aqueous hydrolysis of the borate esters the crude 103 and 107 were isolated. This mixture was "pyrolysed" at 230°C (0.25-0.3 Torr) for 30 minutes. The "distilled" material was subjected to flash chromatography to yield a mixture of 13 and 108. This mixture was again treated with boron tribromide (vide supra) to yield pure 13. In this way reproducible yields (29%: 96 mg of 13 from 506 mg of 77; 31%: 0.351 g of 13 from 1.705 g of 77) were obtained over the three-step sequence.

An attempt to carry out the cyclisation of 103 to 13 by heating 103 in a high boiling solvent (xylenes, bp 136-145°C) consumed all of the starting material but failed to give any detectable amount of 13.



109

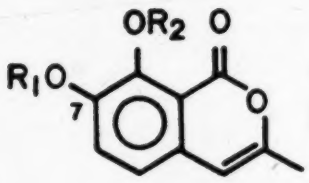
It was thought that the sensitivity of ketone 77 to treatment with more than one equivalent of boron tribromide (vide supra) could be ascribed to the reactivity of the doubly-activated benzylic methylene group. Some negative evidence in support of this hypothesis was obtained by the reaction of benzamide 62 (which has no sidechain) with 2.1 equivalents of boron tribromide. The diphenolic benzamide 109 was isolated cleanly and in high yield (96%). The

frequency (1580 cm^{-1}) of the C=O absorption in the ftir of 109 shows that the amide carbonyl is hydrogen-bonded to the ortho phenol, and is therefore held in one conformation. A consequence of this single, symmetrical conformation is that no diastereo-isomerism is possible about the carbon-nitrogen bond, which in amides has some degree of double bond character. Thus in the ^1H nmr spectrum of 109 the two methyl groups give only a single triplet (δ1.29) and the two methylene groups a single quartet (δ3.55).

The appearance of C=O absorption at low wavenumbers (1580 cm^{-1}) in the ir spectrum of 103 indicates that this amide carbonyl is also hydrogen-bonded to the ortho phenol. Besides eliminating the possibilty of cis/trans amide isomerism, the single conformation in 103 also removes the rotational interdependence of the amido and 2-propanoyl substituents, since the benzylic methylene protons appear as a sharp singlet (δ3.51) in the ^1H nmr spectrum (compare with ketone 77 above).

Isocoumarin 13 was to be elaborated to compounds 14 and 15. Toward 14, a literature[84] precedent was followed. Thus 13 was benzylated (benzyl bromide, potassium carbonate, dry acetone, 56°C for 48 hours). A 4:1 (32%:8%) regioselectivity was demonstrated for alkylation of the phenol at C-7. Compounds 110 and 111 were easily distinguishable on the basis of their ^1H nmr spectra. The chelated phenolic proton of the former appears at δ11.24, while the the latter displays a phenolic proton at δ5.87.

The dibenzylated compound 112 was also obtained (21%).



110 R_1 = Bn R_2 = H

111 R_1 = H R_2 = Bn

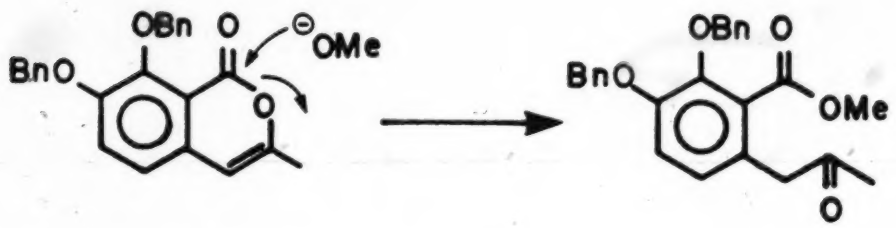
112 R_1 = R_2 = Bn

Formation of the lactol required opening of the unsaturated lactone ring to the corresponding keto-benzoate under basic conditions, followed by careful acidification to avoid dehydration.

This was first attempted by heating compound 112 for 30 minutes with aqueous sodium hydroxide (0.02 N). However, since 112 is dibenzylated, no acidic phenolic proton is available to allow formation of a water-soluble salt. Therefore 112 remained largely undissolved and did not react. The reaction was repeated using a small amount of dimethoxyethane to help solubilise the starting material, but again 112 failed to react. The dibenzylated compound was then refluxed in 0.02 N aqueous sodium hydroxide containing added methanol. Following isolation of the crude material, a more polar compound was observed by tlc in addition to starting material 112. Flash chromatography

gave keto-ester 113 in 14% yield. This compound results

Scheme 27 Formation of Keto-ester 113

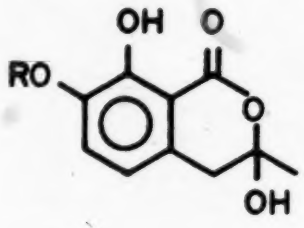


112 113

from opening of the lactone with methoxide ion (Scheme 27). The structure 113 is indicated by the following spectral data. The ir spectrum displays carbonyl absorptions for a ketone (1712 cm^{-1}) and an aromatic ester (1726 cm^{-1}). The methyl group of the ester appears at δ3.77 (3H, s) and the methyl group of the ketone at δ2.13 (3H, s) in the ^1H nmr spectrum. The benzylic methylene group gives a signal at δ3.66 (2H, s). The hreims shows a parent peak at m/z 404 (4% intensity).

Monobenzylated 110 was better suited to preparation of the lactol. Compound 110 was dissolved in 0.02 N aqueous sodium hydroxide and the solution was boiled for 20 minutes. After cooling to 0°C and adding ether, the aqueous layer was carefully acidified to ca. pH 5.5[84]. Extraction with ether at this pH resulted in isolation of a small amount of crude material containing little or no lactol 114. The pH of the aqueous layer was lowered to ca. 2.5 and extraction was repeated. After careful work-up, lactol 114 was obtained in 88% yield. The structure of 114 is supported by ^1H nmr

singlets for the methyl (δ1.78) and methylene (δ3.17) groups and a parent peak in the ms (m/z 300, 8% intensity).



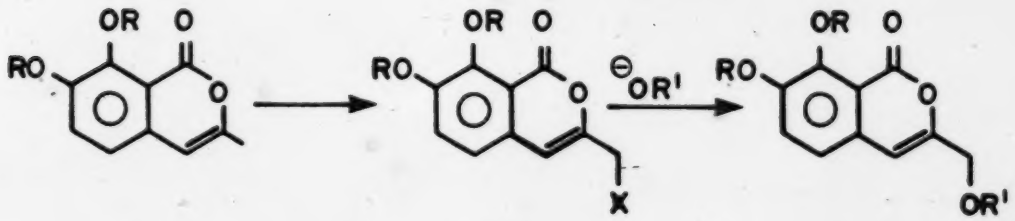
114 R = Bn
14 R = H

Hydrogenolysis (hydrogen, palladium on charcoal, ethyl acetate, 7 hours, room tempetature) of the benzyl group of 114 afforded compound 14 (95%).

Preparation of lactol 14 directly from isocoumarin 13 was also possible. Compound 13 was subjected to the conditions described above for 110. Extraction at pH ca. 5.5 yielded 13 (1%) and 14 (15%), which were separated by flash chromatography. Re-extraction at the lower pH (ca. 2.5) afforded additional 14 (76%) free of any 13. Again caution was exercised not to cause dehydration during the work-up.

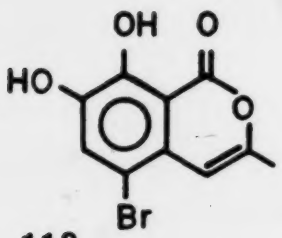
The transformation of 13 into 15 involves the introduction of a hydroxyl group allylic to the carbon-carbon double bond in 13. In order to form the carbon-oxygen bond, an appropriate leaving group (X) had to be installed which subsequently could be displaced by an oxygen-containing nucleophile (Scheme 28). Thus allylic bromination (X=Br) was attempted on isocoumarin 13, using N-bromosuccinimide (NBS) in carbon tetrachloride. Because of the electron donating character of the phenolic substituents, the major product was bromide 118. Location of the bromine at C-5 in based on the observed downfield shifts of the olefinic proton (Δppm = 0.26) and chelated phenolic proton (Δppm = 0.43) from their respective chemical shifts in the ^1H nmr spectrum of 13. An unidentified dibromide was also formed in the reaction. Its molecular formula, $C_{10}H_6O_4Br_2$, is indicated by M+4, M+2, and M^+ peaks

Scheme 28 Creation of an allylic carbon-oxygen bond

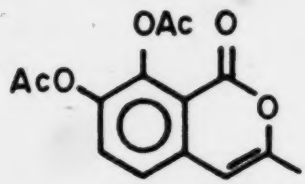


115 116 117

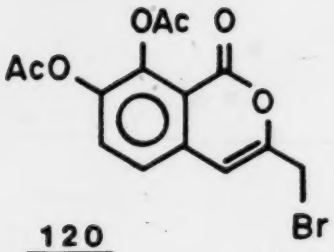
in the ms of 11%, 21%, and 10% intensity respectively.



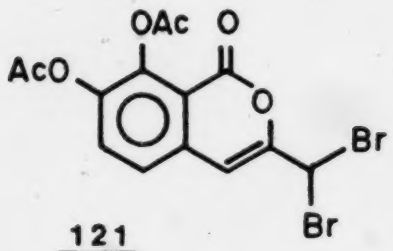
118



119



120



121

To prevent bromination of the aromatic ring, the phenolic hydroxyl groups of 13 were acetylated (pyridine, acetic anhydride, room temperature, 93%) to give compound 119. Before allylic bromination was attempted, 119 was treated with selenium dioxide in refluxing acetic acid to see if the required hydroxyl group could be introduced directly. The starting material was not completely consumed and it was shown by ^{1}H nmr that the unidentified product obtained does not possess the structure of the desired compound.

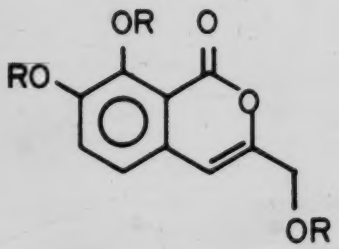
Bromination (NBS) of the diacetate 119 occurred at the allylic position. In an initial effort, the reaction was allowed to proceed for 1.25 hours using 1.0 equivalent of NBS. The presence of bromide 120 was indicated in the ^{1}H

nmr spectrum of the crude material, but starting material 119 still predominated (1.5:1). This material was resubjected to the reaction conditions, employing 2.5 equivalents of NBS for 1 hour. The ^{1}H nmr spectrum of the crude products reveals that 119 had been consumed and bromide 120 was the major component (67%) of the mixture. However, dibromide 121 was also present (33%). The structure of 120 is supported by the appearance of singlets (^{1}H nmr) at δ4.20 (2H) for the methylene group bearing bromine, and at δ6.56 (1H) for the olefinic proton (shifted downfield 0.36 ppm from the corresponding signal in 119). The remainder of the ^{1}H nmr spectrum is in accord with the proposed structure. No parent peak is observed in the hreims, but the cims shows signals for M+2+18 (m/z 374, 95%) and M+18 (m/z 372, 100%). The ^{1}H nmr spectrum of dibromide 121 differs from that of 120 in that the olefinic proton is further downfield (δ6.75) and the methine proton of the dibrominated carbon appears at δ6.29. The ms shows the expected 1:2:1 ratio of intensities for M+4, M+2, and M^+.

Since starting material 119 and product 120 have the same R_f value (0.50), it was not possible to detect the diappearance of 119 by tlc. However, dibromide 121 is slightly less-polar (R_f 0.59), and so it was decided to terminate the reaction at the first appearance of 121. Thus diacetate 119 was treated with NBS (1.1 equivalents) in carbon tetrachloride for six hours. At this stage the crude product was composed of a *ca.* 1:1 mixture of 119 and 120 (^{1}H

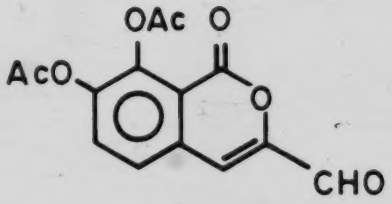
nmr). This material was resubjected to allylic bromination using 1.1 equivalents of NBS. After two hours the dibromide 121 was evident by tlc and the reaction was stopped. Examination by ^{1}H nmr indicated a 2:8.5:1 mixture of 119, 120 and 121. Thus starting material 119 is not completely consumed before dibromide 121 begins to form. Since separation of the components appeared impractical, the mixture of three compounds was used in the next step.

Replacement of Br by OH has been achieved by the use fo silver carbonate[85]. However, this reagent failed to give any reaction when combined with a mixture of bromide 120 and dibromide 121.



122 R = $COCH_3$

15 R = H

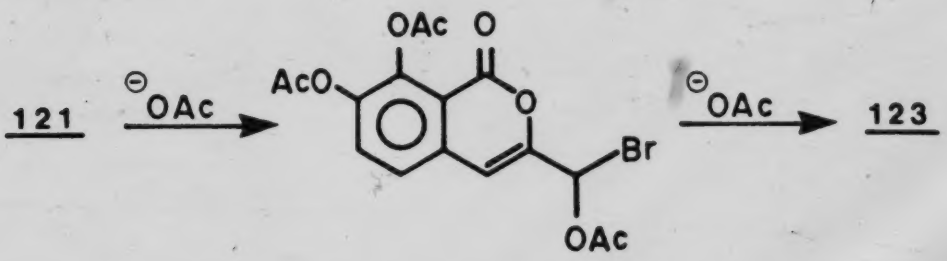


123

A less direct method to substitute the OH group needed in compound 15 for the Br in 120 is *via* the acetate. Silver acetate is well known to effect the displacement of Br by OAc[86]. The method of MacLean[87] was first applied to the *ca.* 2:1 mixture of 120 and 121. Using silver acetate (2.5 equivalents) in refluxing, dry benzene, the reaction proceeded slowly, but after 40 hours the spot corresponding to dibromide 121 had disappeared and two components more

polar than bromide 120 were evident by tlc. The mixture was subjected to flash chromatography to yield recovered 120 (74%), triacetate 122 (27%), and aldehyde 123 (50%). The ^1H

Scheme 29 Formation of aldehyde 123



124

nmr spectrum of 122 shows methyl singlets for the aliphatic acetate (δ2.15) and the aromatic acetates (δ2.35 and 2.42). Singlets appear for the methylene group (δ4.87) and the olefinic proton (δ6.54). The two aromatic protons give rise to doublets (δ7.33 and 7.55, J=8.5 Hz). Aldehyde 123 results from attack of acetate ion on dibromide 121 followed by further reaction of intermediate 124 (Scheme 29). The aldehydic nature of compound 123 is clearly evident from a proton at δ9.59 in the ^1H nmr spectrum. The olefinic proton is far downfield (δ7.25).

The mixture of 119, 120, and 121 (vide supra) was similarly treated with excess (ten equivalents) silver acetate in refluxing, dry benzene. Because the mixture contained 119, it was impossible to distinguish when all of 120 was consumed, since both compounds possess the same R_f (vide supra). After 12 days, more silver acetate (ten

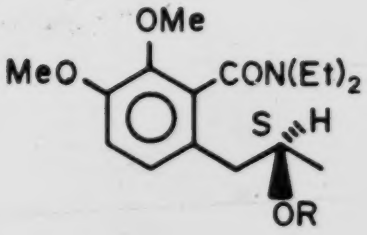
equivalents) was added to the reaction and refluxing was continued for five days. The products were separated by flash chromatography. Diacetate 119 was recovered unchanged (none of 120 was present). Triacetate 122 was obtained in 33% yield over two steps from 119. Aldehyde 123 was also isolated.

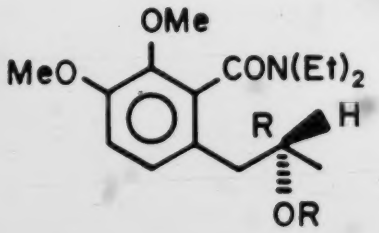
Triacetate 122 was subjected to brief treatment (23 minutes) at room temperature with potassium carbonate in methanol. The products were isolated by acidification and extraction. Dry flash chromatography[88] afforded compound 15 (19%). The low yield of 15 may be partly attributable to its relatively high polarity. Since the compound possesses three hydroxyl groups and a low molecular weight, it may be quite water soluble and have a high affinity for silica gel. The ^1H nmr spectrum of 15 shows the methylene group (2H, s) at δ4.39, shifted upfield by 0.48 ppm (although a difference in solvents also must be taken into account) from the corresponding signal in 122. The alcoholic proton is evident as broad singlet (δ4.66), as are the phenolic protons (δ8.40 and 11.00). The olefinic proton appears as a singlet at δ6.65 and the aromatic protons as doublets (δ6.97 and 7.31, J=8.25 Hz). The hreims gives a parent peak (which is also the base peak) in accord with the structure 15.

Of the compounds 12-15 prepared, only 12 can be chiral. Indeed, the naturally occurring 7-hydroxymellein has $[\alpha]_D$ -97°+/-3°[26]. The R configuration was assigned, presumably by analogy with (R)-mellein, which was isolated from the

same source and possesses optical rotation -108.15°[89]. The R configuration has been determined[90] for the levorotary enantiomer of mellein. In order to firmly establish the configuration of the natural product, we undertook to synthesise both the R and S enantiomers of compound 12.

In parallel experiments, the procedure for the preparation of racemic alcohol 76 was repeated utilising chiral propylene oxide, commercially available in both enantiomeric forms. As in the case of 76, the chiral alcohols (126 and 128) were purified as the corresponding acetates 125 and 127. Starting material 62 and phthalides 88 and 89 were again isolated during purification. On the "R side" an additional by-product, acetate 86, was isolated. As proposed earlier, the phenolic precursor (64) of this





125 R = Ac $[\alpha]_D$ − 1.7° 127 R = Ac $[\alpha]_D$ +0.8°

126 R = H $[\alpha]_D$ +53.7° 128 R = H $[\alpha]_D$ −47.8°

compound probably resulted from exposure of the ortho-metalated species 67 to oxygen. The low yield of (R)-alcohol 128 (37%) compared to that of the (S)-alcohol 126 (73%) may be due, in part, to the formation of phenol 64. The optical rotation of the 128 is somewhat smaller in

magnitude than that of 126. This is attributed, at least in part, to the use of (R)-propylene oxide which was not as optically pure as the (S) enantiomer.

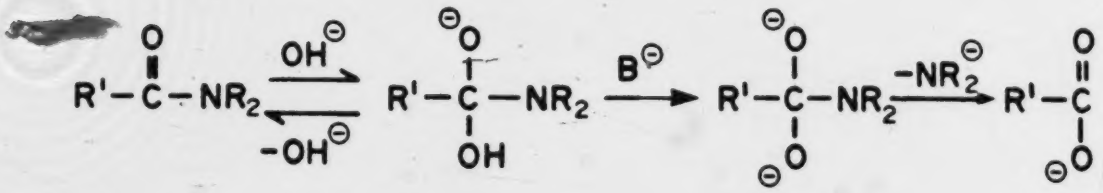
With the chiral centre incorporated, a method was needed to hydrolyse (or otherwise eliminate) the amide moiety without disturbing the chiral carbon. With most previous compounds containing an amide, hydrolysis had been carried out under vigorous, acidic conditions (vide supra). However, such treatment was not appropriate for the chiral alcohols since it could result in dehydration and loss of chirality.

An alternate approach which was explored is hydrolysis under basic conditions. When alcohol 76 (used in trials to conserve chiral compounds) was treated with refluxing, aqueous 3 N sodium hydroxide, small amounts (2-10%) of dimethoxy lactone 75 were isolated, along with starting material, after acidification and extraction. Compound 75 was demethylated with boron tribromide to give 12, identical (tlc, ^{1}H nmr) with 12 prepared by the allylbenzamide (56) route (vide supra). In the basic hydrolysis of 76, it was observed that unless a sufficiently vigorous reflux (i.e. sufficiently hot heating mantle) was employed, no detectable amount of 75 was formed. A variac setting of at least 75 volts was required. When hydrolysis was attempted using potassium hydroxide in refluxing diethylene glycol (bp 240-250°C) neither the starting material 76 nor the desired product 75 could be detected (tlc) in the crude material

isolated.

The difficulty with simple basic hydrolysis of amides using OH^- is the tendency of the tetrahedral intermediate 129 (Scheme 30) to return to starting material rather than to expel amide (NR_2^-), which is a poorer leaving group than

Scheme 30 **Basic amide hydrolysis**



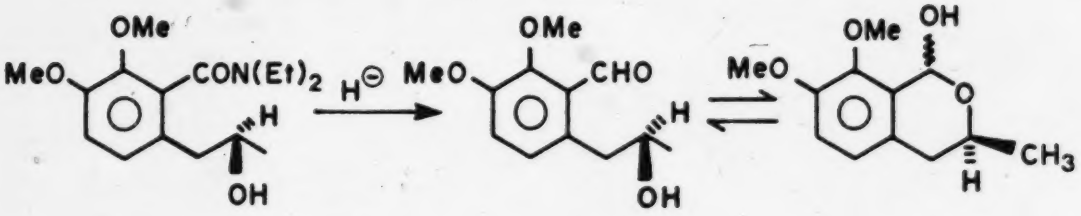
129 **130**

OH^-. However, when it is possible to abstract the OH proton of 129 with a second strong base (B^-) to give intermediate 130, then loss of NR_2^- becomes the thermodynamically preferred pathway. Such a procedure has been reported[91]. It utilises a slurry of potassium t-butoxide (0.122 M), water (0.037 M), and the amide (0.0185 M) in ether at room temperature. However, (S)-alcohol 126 gave no reaction when treated under the above conditions for 24 hours. Dry tetrahydrofuran was substituted for ether and the reaction mixture was refluxed for 48 hours, but 126 failed to react. When refluxing dioxane was used as solvent, a complex reaction mixture (tlc) was obtained which did not contain the desired lactone.

Amides can be reduced to aldehydes by treatment with a single equivalent of complex hydride[92]. Reduction of the

amide to the aldehyde level in 126 would not affect the chirality of the alcohol (Scheme 31). Alcohol 76 was treated with lithium aluminum hydride in dry tetrahydrofuran under progressively harsher conditions. However, even using excess (five equivalents) reducing agent and elevated

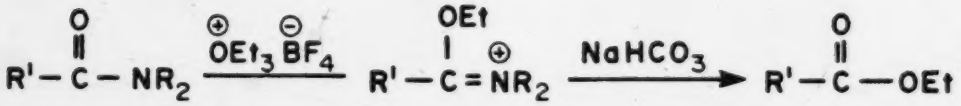
Scheme 31 Reduction of amide to aldehyde



126

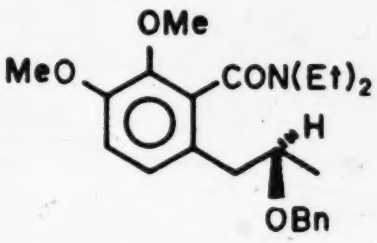
temperature, no reduction of 76 was observed (tlc).

Scheme 32 O-alkylation of amide



Powerful alkylating agents such as Meerwein's reagent[93] are capable of O-alkylation of amides to give imino salts, which may be hydrolysed to esters under mild conditions (Scheme 32). Before attempting O-alkylation of the amide function in 126, the alcohol was protected. Thus benzyl ether 131 was formed by reaction of 126 with sodium hydride and benzyl bromide in dry tetrahydrofuran. Compound 131 was

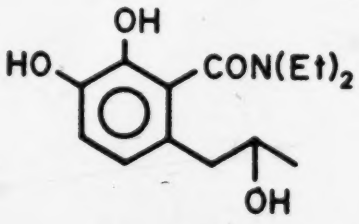
obtained in 92% yield. However, treatment of 131 with



131

trimethyloxonium tetrafluoroborate in dry methylene chloride followed by aqueous potassium bicarbonate led to the reisolation of starting material.

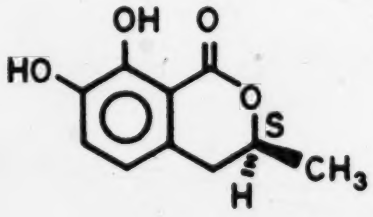
With the intent of facilitating hydrolysis under basic conditions (vide supra) by making the substrate more soluble in aqueous base, alcohol 76 was treated with boron tribromide. As in the case of ketone 77, the reaction did not proceed cleanly. The presumed product, hydroxy diphenol 132, was difficult to purify and thus was set aside. After some three months, a small sample of the reaction product



132

was reexamined by tlc. A previously absent, less polar compound appeared to be present in considerable quantity. Upon separation by flash chromatography this new component

proved to be lactone 12. The reaction with boron tribromide was repeated using chiral alcohol 126. By tlc it was clear that the freshly isolated crude reaction product did not contain 12. However, after the crude product had been standing in chloroform at room temperature for a few hours, compound 12 was evident (tlc). On the other hand, no trace of 12 was observed (tlc) when crude product (27 mg), dissolved in deuterated chloroform (ca. 0.5 mL), was stored at ca. 5°C for 24 days. The dry crude product could be stored in vacuo for indefinite periods without any detectable (tlc) formation of 12. The amount of lactone generated was about the same (39% vs. 33% from 126) whether a stirred (room temperature), chloroform solution of crude was irradiated with a light source (100 watt light bulb) for a week, or kept in the dark for the same period of time. The yield of lactone was higher (50%) when a chloroform solution of the crude was refluxed for 48 hours exposed to ordinary light. The optical rotation of lactone arising from 126 was positive, indicating the S configuration (133).

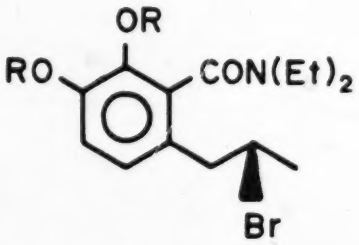


133

However, the magnitude of the rotation was smaller ($[\alpha]_D$ +45.8°) than would be anticipated for optically pure

(S)-7-hydroxymellein (vide supra). Therefore it appeared that some racemisation was taking place during the two step process from 126 to 12. In order to understand this sequence, in which amide hydrolysis was occurring under very mild conditions, it was necessary to prove the structure of intermediate 132.

The crude product of the boron tribromide reaction was acetylated (pyridine, acetic anhydride). The product was purified by flash chromatography. Its ^1H nmr spectrum indicates the presence of only two acetyl groups, since singlets for the acetyl methyls integrate for only six protons. The chemical shift of the methine proton resonating at δ4.37 is almost unchanged from its location (δ4.33) in the parent diphenol. Signals appear in the hreims at m/z 415 and 413. Their equal intensities (0.4%) suggest the inclusion of a bromine atom in the molecular formula. That these signals represent the parent peaks is

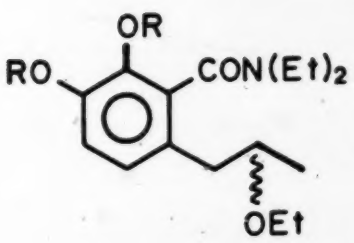


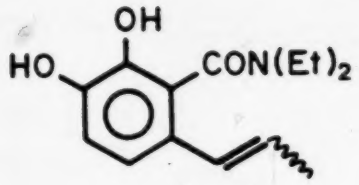
134 R = $COCH_3$
135 R = H

confirmed by cims (m/z 433 (415+18), 98% and 431 (413+18), 100%). These data led to the assignment of 134 as the structure of the acetylation product. Therefore the product

of boron tribromide treatment of alcohol 126 is the bromide 135.

Further experiments were performed to acquire more evidence in support of structure 135. The reaction of acetate 79 with boron tribromide gave a crude product equivalent to 135 in tlc behaviour. The hreims and ^1H nmr spectrum also contained the same major signals as those of 135.





136 R = H

137 R = $COCH_3$

138

Following the method of Cromwell[94], treatment of 135 with an ethanolic solution of silver acetate (1.1 equivalents) resulted in the immediate precipitation of silver bromide. The salt was isolated by filtration in 81% yield from 126. Flash chromatography of the other products afforded lactone 133 (stereochemistry assumed) in 31% yield from 126. The structure of a second product (40% from 126) is tentatively assigned as ethyl ether 136 on the basis of hreims, which shows major peaks at m/z 295 (M^+, 42%), 223 ($M^+ - CH_2{=}CH(OEt)$, 46%), and 208 ($M^+ - CH_2CH(OEt)CH_3$, 73%). Compound 136 was acetylated (pyridine, acetic anhydride) to give diacetate 137, the hreims of which is also in agreement

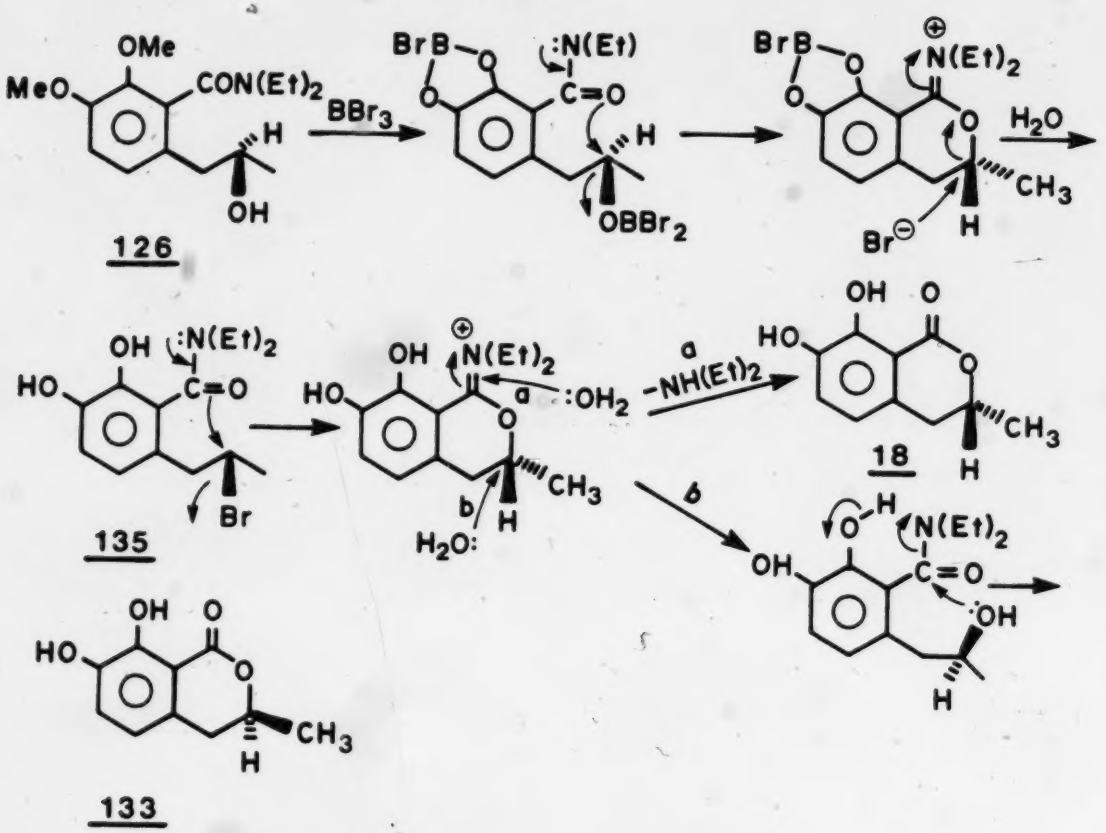
with the assigned structure.

The reaction of crude bromide 135 with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 3.1 equivalents) in refluxing benzene led to the formation of a 2:1 mixture of lactone 133 (stereochemistry assumed) and olefin 138. The structure of the latter compound is indicated by a methyl doublet (δ1.81, J=6.25 Hz) and olefinic protons (δ6.03 and 6.15) in the ^{1}H nmr spectrum.

Since the optical rotation of crude 135 is positive ($[\alpha]_D$ +54.6°), most of the bromide presumably possesses the S configuration ((S)-alcohol 126 $[\alpha]_D$ +53.0°). In order to explain the observed retention of configuration from 126 to 135 to 133, the proposed mechanism in Scheme 33 invokes participation of the amide. Partial racemisation would occur via pathway "a". On the other hand, the positive rotation of crude 135 does not prove that it has the S configuration. If the configuration is actually R, then the sequence should involve inversion from 126 to 135 (displacement by Br^-), and inversion again from 135 to 133 (invoking participation of the amide carbonyl as in Scheme

33).

Scheme 33 Formation of **133** from **126**

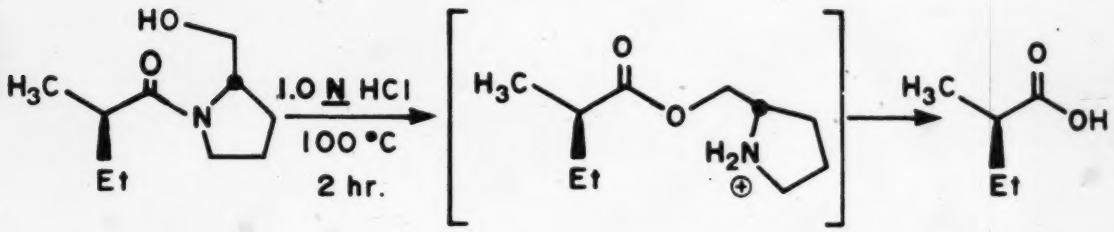


Although the formation of lactone **133** from bromide **135** is an unusual transformation, the loss of integrity at the chiral centre made it an unattractive route for the preparation of optically pure enantiomers of lactone **12**.

In spite of earlier concerns about dehydration of alcohols **126** and **128**, acid hydrolysis of the amide function was reconsidered. Evans[95] has employed a proximal hydroxyl group to facilitate amide hydrolysis under relatively mild conditions (Scheme 34). This nitrogen-to-oxygen acyl

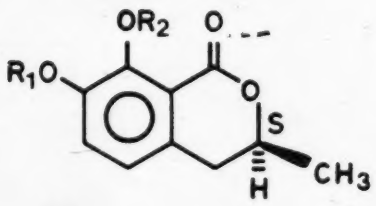
transfer proceeds through a five-membered transition state.

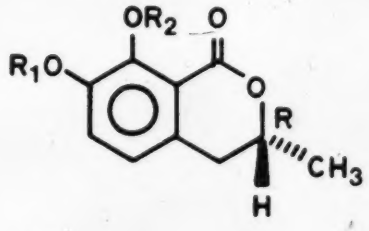
Scheme 34 Evans' amide hydrolysis



139 **140** **141**

Encouraged by this precedent, we attempted formation of dimethoxy lactone 75 by amide hydrolysis of alcohol 76. Transacylation also should be favourable in this reaction since it involves a six-membered transition state. A solution of 76 in 1 N hydrochloric acid was refluxed for two hours. Some of 75 was obtained, but it was isolated in very





142 $R_1=R_2=CH_3$ $[\alpha]_D$ +146.3° **144** $R_1=R_2=CH_3$ $[\alpha]_D$ −129.3°

143 $R_1=CH_3$ $R_2=H$ **145** $R_1=CH_3$ $R_2=H$

133 $R_1=R_2=H$ $[\alpha]_D$ + 92.0° **18** $R_1=R_2=H$ $[\alpha]_D$ −88.6°

low yield (8%) as a 1:1.5 mixture with allylbenzamide 56. Compounds 56 and 75 are practically inseparable by tlc. A

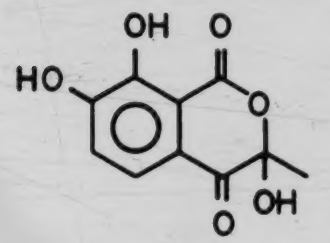
large amount (79%) of starting material 76 was also recovered. It was noted that Evans' conditions specify heating at 100°C, not under reflux. Therefore the reaction was repeated using chiral alcohol 126, 1 N hydrochloric acid, and a 100°C constant temperature oil bath. A longer reaction time (24 hours) was employed in light of the low yield of 56 and 75 obtained in the previous reaction. Lactone 142 was isolated in 49% yield (79% based on consumed starting material) along with recovered alcohol 126 (38%). A very small amount of a mixture of monomethoxy lactone 143 (stereochemistry assumed) and phthalide 88 was also obtained. Significantly, no olefinic material was detected.

The procedure was also performed on (R)-alcohol 128 and gave a 51% yield of 144 (91% based on consumed starting material), recovered 128 (44%), and a small quantity of a 5:1 mixture of 145 (stereochemistry assumed) and 88.

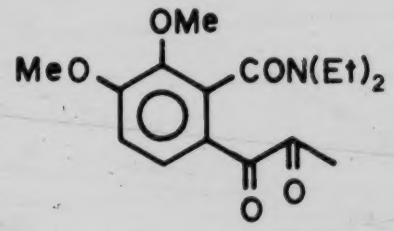
Each of the two dimethoxy lactones, 142 and 144, was converted into the corresponding diphenolic lactone (133 and 18). The yields were quantitative. Since none of the synthetic steps from the chiral propylene oxides to 133 and 18 involve an inversion of configuration, the naturally occurring compound must have the R stereochemistry. The small difference (5°) in magnitude of optical rotation between 133 ((S)-7-hydroxymellein) and natural 7-hydroxymellein suggests that the synthetic material possesses a high degree of optical purity. The rotation of the synthetic (R)-isomer is lower, presumably due, at least

in part, to partly racemic (R)-propylene oxide (vide supra).

Our approach to keto-lactol 17 involved the establishment of the correct level of oxidation in the three-carbon sidechain. Since α-diketone 105 fulfills the oxidation requirement, its preparation was investigated.



17

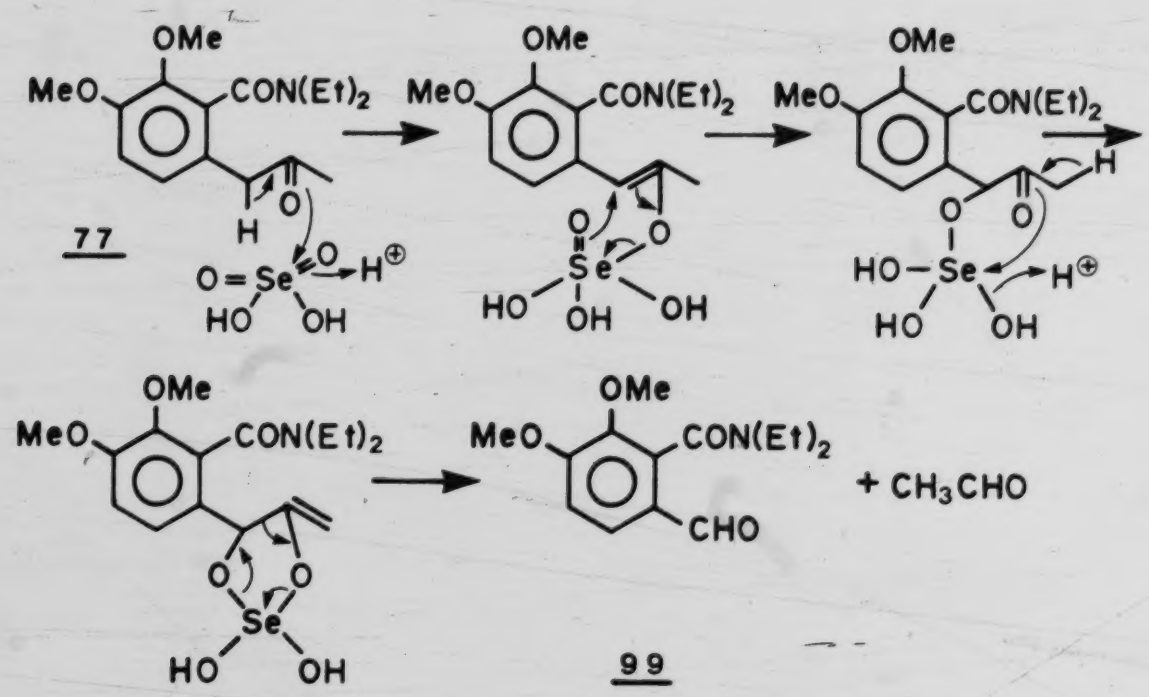


105

Selenium dioxide oxidation is a common method for the formation of α-diketones from ketones[96]. Ketone 77 appeared to be a likely candidate for this reaction since the desired site of oxidation is activated not only by the ketone but also the aromatic ring. The reaction of 77 with selenium dioxide was attempted in several solvents, including acetic acid-water (7:3), 95% ethanol, and dry pyridine. In all cases complex mixtures of products were obtained. The only characterisable compound isolated (in low yield) was aldehyde 99, the product of carbon-carbon bond cleavage. Its formation is mechanistically rationalised in Scheme 35. Since this approach to 105 did not appear promising, an

alternate route was explored.

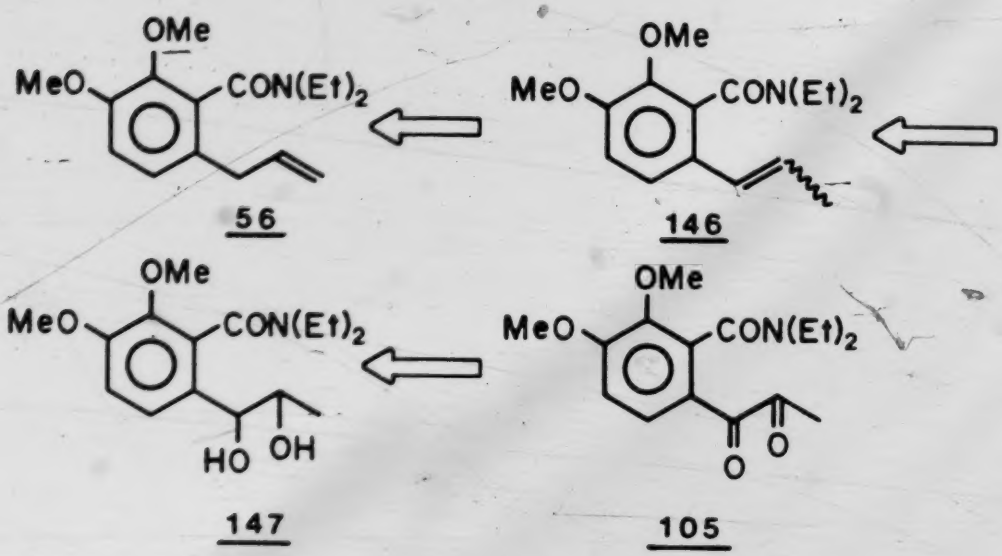
Scheme 35 Formation of aldehyde **99**



Scheme 36 shows retrosynthetically how α-diketone could arise from allyl benzamide 56. The formation of olefin 146 from 56 required isomerisation of the carbon-carbon double bond. This should be a thermodynamically favourable process since in 146 the double bond is in conjugation with the aromatic ring. The isomerisation was attempted using para-toluenesulphonic acid (pTsOH). After refluxing a solution of 56 and pTsOH (0.1 equivalent) in toluene for 17.5 hours, no reaction had occurred. Compound 56 was resubjected to the reaction conditions using 1.0 equivalent of pTsOH. The reaction proceeded very sluggishly, and after

five days an additional equivalent of pTsOH was added.

Scheme 36 Retrosynthetic route, 105 to 56

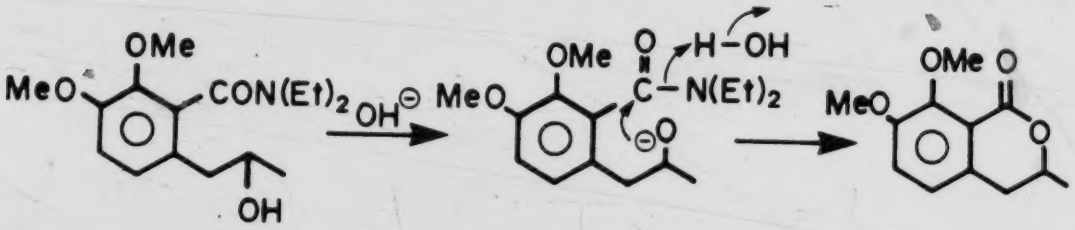


Reflux was continued for two days. Examination of the crude reaction products by ^{1}H nmr showed that starting material 56 was still present in excess of 146. Phthalide and lactone 75 were among the other products.

The isomerisation of 56 to 146 was found to proceed cleanly under basic conditions. Thus 56 was dissolved in methanolic 3 N sodium hydroxide and the solution was refluxed for 68 hours. Olefin 146 was isolated in 99% yield as a 7:1 (trans-cis) mixture of isomers. The reason for preferred formation of the trans carbon-carbon double bond is presumably a steric one. The vinylic methyl group experiences much more serious steric interactions with the amido functionality in the cis compound than in the trans.

The two isomers, which are inseparable by tlc, were identified by analysis of the ^{1}H nmr of the mixture. The olefinic protons of the major (trans) isomer have a large (15.75 Hz) vicinal coupling constant, while the olefinic protons of the minor (cis) isomer show a smaller (11 Hz) vicinal coupling.

Scheme 37 Formation of 75 under basic conditions

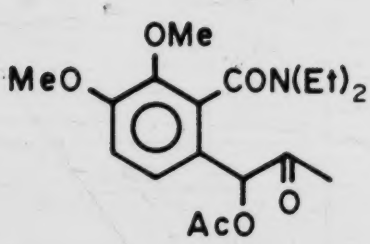


76 148 75

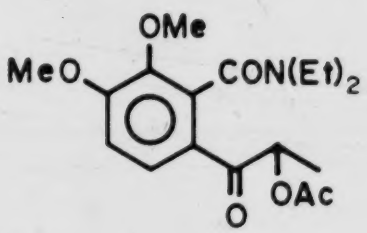
For the purpose of comparison, allylbenzamide 56 and alcohol 76 were treated with aqueous 3 N sodium hydroxide under identical reaction conditions. Compound 56 gave only 146 (i.e. no hydrolysis occurred), and 76 gave a small amount (3%) of hydrolysis product 75. This result indicates that in the case of alcohol 76, the hydroxyl group may serve as an intramolecular nucleophile (148, Scheme 37).

Transformation of an olefin into a glycol is usually carried out by oxidation with osmium tetroxide[97] or cold, alkaline potassium permanganate[98]. Such reagents might have been employed in the preparation of diol 147 (Scheme 36) from olefin 146. The diol then would have been converted to the α-diketone 105 using a method such as the Swern oxidation[83]. However, compound 147 was by-passed in favour

of the direct formation of 105 from 146 using the conditions described by Sharpless[99]. When the literature procedure was followed exactly, α-diketone 105 was obtained in 15% yield after flash chromatography. Keto-acetate 150 was also isolated (1%). In a repeat reaction, the aqueous phase of



149



150

the reaction mixture was thoroughly extracted. The yield of 105 was improved to 41%. Compound 150 was obtained in 7% yield and keto-acetate 149 (4%) was also isolated. Isomeric compounds 149 and 150, each of which exist as a pair of rotamers, are easily distinguishable by ^{1}H nmr. The methine proton of 149, having no coupling partners, appears as singlets (δ5.89 and 5.91), whereas the methine proton of 150 is split to quartets (δ5.84 and 5.91, J=7 Hz) by the methyl group. Likewise, the sidechain methyl group in 149 gives rise to two singlets (δ2.15 and 2.18), while in 150 a single doublet (δ1.47, 6H, J=7 Hz) appears for the sidechain methyl. The diketone itself is typified by its bright yellow colour. Its spectral data, including a methyl singlet at δ2.47 (^{1}H nmr) and carbonyl resonances at δ190.5 and 199.9 (^{13}C nmr), are in full agreement with structure

105.

Acid hydrolysis (6 N hydrochloric acid, reflux) of the amide of 105 did not give characterisable products. Treatment of 105 with boron tribromide followed by distillation of the crude reaction products gave a very complex mixture of compounds. The route from 105 to 17 was not further investigated.

The ortho-acyl-substituted catechols synthesised in this study include the 7,8-diphenolic analogues (12-15) of the Ceratocystis metabolites 6-9, 6-hydroxyisoochracin (70), and both (S)- (133) and (R)-7-hydroxymellein (18), the latter having the same configuration as the natural product. Compounds 12-15 gave blue- to green-coloured complexes when combined with 1% (w/v) ferric chloride in methanol.

Since one acyl substituent confers significant iron-binding capability upon a catechol moiety, additional acyl groups should enhance siderophoric activity. For this reason, keto-lactol 17 may be the best siderophore of 12-17, and thus its synthesis may merit further research.

With several substrates now available from this and other studies, the iron-binding capacity and *in vivo* activity of these compounds will be determined. Diphenolic benzamide 109 should be included in this testing since many naturally occurring catechol-type siderophores possess an amide function as the ortho-acyl substituted[100]. The N,N-dimethyl amido derivative of 109 has been the subject of a study on siderophore analogues[101]. Preliminary bioassays

on 7-hydroxymellein (12) indicate that it may have activity in relation to the water transport system of lodgepole pine[102]. Further bioassays may help establish what role, if any, the iron-complexing metabolites of the blue stain fungi play in the cause of the disease. Such knowledge could provide a basis from which a control of the blue stain disease may arise.

3.2 EXPERIMENTAL

General Unless specified, all solvents, with the exception of nitromethane, ether, dioxane, and cyclohexane, were distilled prior to use. Skellysolve B refers to Skelly Oil Company light petroleum, bp 62-70°C. Anhydrous solvents and reagents were distilled from appropriate drying agents (in brackets): tetrahydrofuran (potassium), methylene chloride (calcium hydride), acetone (calcium sulphate), ether (sodium), benzene (sodium), carbon tetrachloride (3 A molecular sieves), pyridine (calcium hydride), propylene oxide (calcium hydride), 1,2-dibromoethane (calcium chloride), triethylamine (calcium hydride) and N,N,N',N'-tetramethylethylenediamine (calcium hydride). E. Merck silica gel 60 (230-400 mesh) was used for flash chromatography[35]. Preparative thin layer chromatography (ptlc) was carried out on Terochem silica gel G containing a trace of electronic phosphor (General Electric), 20x20, 10x20, or 5x20 cm plates, 0.35 mm thickness. Analytical thin layer chromatography (tlc) was done on E. Merck precoated plates of silica gel 60 F-254 (0.2 mm thickness). All R_f values are for single elution unless otherwise indicated. Ultraviolet active materials were detected by visualisation under a uv lamp (254 or 350 nm). The plate (only a thin vertical band in the case of ptlc) was then sprayed with either a solution of 30% (v/v) sulphuric acid or 3% (w/v) phosphomolybdic acid in 5% (v/v) sulphuric acid

containing a pinch of ceric sulphate, and charred on a hot plate.

High resolution electron electron impact mass spectra (hreims) were recorded on an AEI MS-50 mass spectrometer. Chemical ionisation mass spectra (cims) were obtained using an AEI MS-12 mass spectrometer. The data were processed in DS-55 and Nova-4 computers. Fourier transform infrared (ftir) spectra were recorded as $CHCl_3$, CH_2Cl_2, CH_3COCH_3, or CH_3OH casts on a Nicolet 7199 FTIR interferometer. Single scan infrared (ir) spectra were recorded on a Perkin Elmer 297 spectrometer. Nuclear magnetic resonance (nmr) spectra (^1H and ^{13}C) were obtained on Bruker WH-200 or WH-400 spectrometers with an Aspect 2000 computer system. Tetramethylsilane was used as the internal standard. Ultraviolet (uv) spectra were obtained on a Unicam SP 1700 spectrophotometer or a Hewlett Packard 8450A Diode Array spectrophotometer. Optical rotations were measured on Perkin Elmer 141 or 241 polarimeters. Melting points are uncorrected and were determined on Zeitz-Wetzlar and Thomas Model 40 melting point apparatus. Elemental analyses were carried out by the microanalytical laboratory of this department.

2,3-dimethoxybenzaldehyde (60)

A magnetically stirred solution of ortho-vanillin (59, 25.0 g, 0.164 mol), potassium carbonate (31 g, 0.224 mol), and methyl iodide (65 mL, 1.05 mol) in dry acetone (200 mL)

was heated at 45°C in a constant temperature oil bath for 48 hours. Upon cooling to room temperature the solution was filtered through a Celite pad and the filtrate was evaporated to dryness *in vacuo* to afford crude material (30.7 g). Flash chromatography (Skellysolve B-ethyl acetate, 93:7; sample dissolved in chloroform; 4.5 cm column) in two portions gave the pure aldehyde **60** (26.0 g, 96%). An analytical sample was obtained by recrystallisation from ether (mp 49-51°C)(lit.[66] mp 50-52°C); tlc: R_f 0.40 (Skellysolve B-ethyl acetate-acetic acid, 85:15:1); ftir($CHCl_3$ cast): 1689 cm^{-1}; uv(cyclohexane): 228(ϵ12,300), 253(ϵ11,000), 259(ϵ10,750), 315(ϵ3350)nm; hreims: m/z calcd. for $C_9H_{10}O_3(M^+)$: 166.0630; found 166.0630(100), 151(21), 108(20), 105(21), 95(62), 93(37), 90(36), 80(22), 77(37), 76(29); ^1H nmr($CDCl_3$): δ3.94(3H, s, OCH_3), 4.01(3H, s, OCH_3), 7.16(2H, m, 2x ArH), 7.44(1H, dd, J=6, 3.5 Hz, ArH), 10.47(1H, s, CHO); ^{13}C nmr($CDCl_3$): δ56.0, 62.2(CH_3); 118.2, 119.2, 124.0(CH); 129.8, 152.7, 153.0(C); 189.9(C=O); Analysis: calcd. for $C_9H_{10}O_3$: C 65.05, H 6.07; found C 64.73, H 5.99.

2,3-dimethoxybenzoic acid (**58**)

Benzaldehyde **60** (31 g, 0.187 mol) and potassium bicarbonate (36 g, 0.36 mol) were dissolved in magnetically stirred, refluxing water (300 mL). *Via* a dropping funnel, a hot aqueous solution of potassium permanganate (23 g, 0.146 mol) was added gradually. When the addition was complete

the solution was further refluxed for one hour. After allowing the reaction mixture to cool, it was filtered, and the brown residue (manganese dioxide) was washed with hot water. The filtrate was acidified to pH 3 with 10% hydrochloric acid (v/v) and evaporated to dryness in vacuo. Inorganic salts were removed by dissolution of the crude product in acetone followed by filtration. Removal of the acetone by evaporation in vacuo afforded 58 (34 g, quantitative). An analytical sample was obtained by recrystallisation from 95% ethanol (mp 120-122°C)(lit.[67] 120-122°C).tlc: R_f 0.14 (Skellysolve B-ethyl acetate-acetic acid, 85:15:1); ftir(CH_3OH cast): 3500-2400(b), 1701, 1686, 1500, 1319, 1267, 1054, 760 cm^{-1};uv(CH_3OH): 220(ϵ11,500), 243(sh.)(ϵ3600), 295(ϵ2300)nm; hreims: m/z calcd. for $C_9H_{10}O_4(M^+)$: 182.0579; found 182.0582(100), 149(33), 137(24), 109(26), 107(72); ^1H nmr(acetone-d_6+D_2O): δ3.77(3H, s, OCH_3), 3.78(3H, s, OCH_3), 7.06(1H, t, J=7.75 Hz, ArH), 7.15(1H, dd, J=7.75, 2 Hz, ArH), 7.25 (1H, dd, J=7.75, 2 Hz, ArH); ^{13}C nmr(acetone-d_6+D_2O): δ56.4, 61.6(CH_3); 117.0, 122.6, 124.8(CH); 126.4, 149.0, 153.8(C); 168.6(C=O); Analysis calcd. for $C_9H_{10}O_4$: C 59.34, H 5.53; found C 59.25, H 5.50.

2,3-dihyroxybenzoic acid (4, hydrobromic acid route)

Dimethoxybenzoic acid (58, 0.254 g, 1.40×10^{-3} mol) was treated with hydrobromic acid (47%, 5 mL) and the magnetically stirred solution was refluxed for three hours.

After cooling to room temperature the reaction mixture was diluted with saturated aqueous ammonium chloride (25 mL) and extracted with ethyl acetate (3x 25 mL). The combined organic extracts were dried over sodium sulphate and evaporated to dryness *in vacuo* to give the crude acid **4** (0.227 g). The acid was further purified by dissolving the crude acid in methanol containing Norit A charcoal and boiling for five minutes, then filtering, evaporating to dryness, and recrystallising from water (mp 195-200°C, lit.[66] mp 206-207°C). tlc: R_f 0.14 (Skellysolve B-ethyl acetate-acetic acid, 75:25:1); ftir(MeOH cast): 3500-2400(b), 1677, 1659, 1474, 1299, 1256, 1234, 1159 cm^{-1}; uv(MeOH): 221(ϵ12,500), 248(ϵ6850), 319(ϵ3150)nm; hreims: m/e calcd. for $C_7H_6O_4(M^+)$: 154.0266; found 154.0267(34), 136(100), 108(26), 80(18); ^1H nmr(acetone-d_6+D_2O): δ6.78(1H, t, J=8 Hz, ArH), 7.07(1H, dd, J=8, 1.5 Hz, ArH), 7.38(1H, dd, J=8, 1.5 Hz, ArH); ^{13}C nmr(CD_3OD): δ119.7, 121.5, 121.9(CH); 114.0, 146.6, 151.4(C); 173.7(C=O).

2,3-dihydroxybenzoic acid (**4**, boron tribromide route)

To a magnetically stirred solution of boron tribromide in methylene chloride (5 ml, 1 g/5 mL) was added dimethoxybenzoic acid (**58**, 0.255 g, $1.40x10^{-3}$ mol) dissolved in methylene chloride (5 mL). The reaction was stirred overnight at room temperature, then water (10 mL) was added, and stirring was continued for an additional 30 minutes. The product was isolated by addition of saturated aqueous

ammonium chloride (25 mL), extraction with ethyl acetate (3x 25 mL), drying of the organic layers over sodium sulphate, filtration, and evaporation *in vacuo* to afford the crude product (0.252 g). Recrystallisation from water gave acid **4** (0.164 g, 76%).

2,3-dimethoxybenzoic acid chloride (**61**)

Dimethoxybenzoic acid (**58**, 0.269 g, 1.48×10^{-3} mol) was dissolved in dry benzene (20 mL) and oxalyl chloride (2.0 mL, 2.36×10^{-2} mol) was added. The magnetically stirred solution was refluxed for one hour. Upon cooling to room temperature the reaction mixture was evaporated to dryness *in vacuo* to afford crude, crystalline **61** (0.280 g, 94%; mp 45-47°C) which was stored under vacuum.*. tlc: R_f 0.27 (Skellysolve B-ethyl acetate-acetic acid, 75:25:1)(some hydrolysis apparent on silica gel); ir(Nujol): 1779 cm^{-1}; uv(cyclohexane): 221(ϵ9000), 246(ϵ3700), 304(ϵ1300)nm; hreims: m/z calcd. for $C_9H_9O_3{}^{37}Cl$(M+2): 202.0211; found 202.0205(1.7); calcd. for $C_9H_9O_3{}^{35}Cl$(M^+): 200.0241; found 200.0238(3.5)(partial hydrolysis to the acid **58** gives rise to $C_9H_{10}O_4$(100)).

*However, after several days under vacuum, the crystalline substance had mp 60-63°C. The hreims gives no M^+ peak for **61** but shows a signal at $C_{18}H_{18}O_7$(14%) indicative of the corresponding anhydride, formed by condensation of the acid chloride **61** with the acid **58**.

N,N-diethyl-2,3-dimethoxybenzamide (62)

Dimethoxybenzoic acid (58, 34 g, 0.187 mol) was dissolved in dry benzene (200 mL). The magnetically stirred solution was cooled to 0°C in an ice bath, and oxalyl chloride (58 mL, 0.69 mol) was added, resulting in vigorous effervescence. After the gaseous evolution subsided the reaction was gradually heated to reflux under an atmosphere of argon. The solution was refluxed for 22.5 hours, then allowed to cool to room temperature. Evaporation to dryness *in vacuo* afforded the crude acid chloride 61, which was re-dissolved in dry benzene (200 mL). After cooling to 0°C in an ice bath, diethylamine (35 mL, 0.34 mol) was added. Under an argon atmosphere, the reaction was stirred for one hour at room temperature, then refluxed for 24 hours. The solution was allowed to cool to room temperature and then evaporated to dryness *in vacuo*. The crude product was purified in portions by flash chromatography (Skellysolve B-ethyl acetate-acetic acid, 65:35:1; sample dissolved in chloroform.)* For use in the next step, the product was further purified by distillation 90-95°C/0.4-0.45 Torr) to afford benzamide 62 as a colourless liquid. tlc: R_f 0.22 (Skellysolve B-ethyl acetate-acetic acid, 65:35:1); ftir($CHCl_3$ cast): 1635, 1580, 1480, 1427, 1266, 1050 cm^{-1}; uv(cyclohexane): 223(ϵ9200), 280(ϵ1500)nm; hreims: m/z calcd. for $C_{13}H_{19}NO_3(M^+)$: 237.1366; found 237.1365(24), 165(100); ^1H nmr($CDCl_3$): δ1.02(3H, t, J=7 Hz, CH_3), 1.22(3H, t, J=7 Hz, CH_3), 3.15(2H, q, J=7 Hz, CH_2), 3.44(1H, m, CH_2),

3.66(1H, m, CH), 3.81(3H, s, OCH_3), 3.83(3H, s, OCH_3), 6.76(1H, dd, J=8, 1.5 Hz, ArH), 6.89(1H, dd, J=8, 1.5 Hz, ArH), 7.04(1H, dd, J=8 Hz, ArH); ^{13}C nmr($CDCl_3$): 12.4, 13.6, 55.4, 61.0(CH_3); 38.6, 42.7(CH_2); 112.3, 118.4, 124.2(CH); 131.9, 144.4, 152.3(C); 168.1(C=O); Analysis: calcd. for $C_{13}H_{19}NO_3$: C 65.80, H 8.07, N 5.90; found C 66.03, H 8.09, N 5.92.

*In a smaller scale reaction (58, 0.660 g), flash chromatography (Skellysolve B-ethyl acetate-acetic acid, 69:30:1; sample dissolved in chloroform; 4.5 cm column) of the crude product yielded benzamide 62 (0.821 g, 95%) as well as recovered starting material (58, 0.018 g, 2%).

N,N-diethyl-2,3-dimethoxy-6-deuterobenzamide (68)

A magnetically stirred solution of dimethoxybenzamide 62 (0.339 g, 1.43×10^{-3} mol), dissolved in dry tetrahydrofuran (20 mL), was cooled to -78°C in a dry-ice bath under an argon atmosphere. TMEDA (0.54 mL, 3.58×10^{-3} mol) and s-butyllithium (1.26 M in cyclohexane, 2.8 mL, 3.58×10^{-3} mol) were added. After 30 minutes the reaction was quenched with excess D_2O (0.5 mL) and the solution was allowed to warm to room temperature. Saturated aqueous ammonium chloride (20 mL) was added and the product was isolated by extraction with ethyl acetate (3x 20 mL). The combined organic layers were dried over sodium sulphate,

filtered, and evaporated *in vacuo* to afford crude deuterobenzamide **68** (0.345 g). tlc: R_f 0.22 (Skellysolve B-ethyl acetate-acetic acid, 65:35:1); ftir($CHCl_3$ cast): 1635 cm^{-1}; hreims: m/z calcd. for $C_{13}H_{18}NO_3D(M^+)$: 238.1428; found 238.1443(31), 166(100); ^1H nmr($CDCl_3$): δ1.03(3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 1.25(3H, t, J=7 Hz, $\underline{CH_2}CH_3$), 3.18(2H, q, J=7 Hz, CH_2), 3.45(1H, m) and 3.7(1H, m, CH_2), 3.84(3H, s, OCH_3), 3.86(3H, s, OCH_3), 6.93(1H, d, J=8 Hz, ArH), 7.10(1H, d, J=8 Hz, ArH).

N,N-diethyl-2,3-dimethoxy-6-allylbenzamide (**56**)

A representative example of a typical run is given. Benzamide **62** (1.53 g, 6.48×10^{-3} mol) was dissolved in dry tetrahydrofuran (40 mL) under an argon atmosphere. The magnetically stirred solution was cooled to -78°C in a dry-ice bath. Following the addition of TMEDA (2.0 mL, 1.30×10^{-2} mol) and *s*-butyl lithium (1.29 *M* in cyclohexane, 10.1 mL, 1.30×10^{-2} mol), the mixture was stirred for 30 minutes. Freshly prepared magnesium bromide etherate[72] (2.62 *N* in ether, 7.4 mL, 1.94×10^{-2} mol) was added, resulting in the formation of a white precipitate. After 40 minutes, allyl bromide (5.6 mL, 6.48×10^{-2} mol) was added, and the solution was allowed to warm to room temperature overnight. The reaction mixture was diluted with saturated aqueous ammonium chloride (40 mL). The products were isolated by ethyl acetate extraction (3x 40 mL). The combined organic layers were dried over sodium sulphate,

filtered, and concentrated to a yellow oil (1.7 g). Flash chromatography (4.5 cm column; sample dissolved in chloroform; Skellysolve B-ethyl acetate-acetic acid, 75:25:1, changing to 65:35:1 after 62 began to elute pure) provided the allyl benzamide 56 (1.347 g, 75%) along with recovered 62 (0.150 g, 10%). The best reproducible yields for this reaction ranged from 61 to 92%. Distillation (124-129°C/0.45-0.5 Torr) gave pure 56, free of any bromide 65 (vide infra). Compound 56 has the following physical properties. tlc: R_f 0.35 (Skellysolve B-ethyl acetate-acetic acid, 65:35:1); ftir($CHCl_3$ cast): 1633 cm^{-1}; uv(cyclohexane): 218(ϵ8800), 283(ϵ1900)nm; hreims: m/z calcd. for $C_{16}H_{23}NO_3(M^+)$: 277.1678; found 277.1682(48), 262(5), 205(100), 177(15); ^1H nmr($CDCl_3$): δ1.05(3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 1.26(3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 3.10(1H, quintet, J=7 Hz) and 3.12(1H, quintet, J=7 Hz, $\underline{CH_2}CH_3$), 3.23(1H, ddt, J=15, 7, 1 Hz) and 3.31(1H, ddt, J=15, 6, 1.25 Hz, $ArCH_2$), 3.54(1H, dq, J=13.5, 7 Hz) and 3.63(1H, dq, J=13.5, 7 Hz, $\underline{CH_2}CH_3$), 3.83(3H, s, OCH_3), 3.86(3H, s, OCH_3), 5.09(2H, m, $CH=\underline{CH_2}$), 5.91(1H, dddd, J=17, 10, 7, 6 Hz, $\underline{CH}=CH_2$), 6.85(1H, d, J=8.5 Hz, ArH), 6.93(1H, d, J=8.5 Hz, ArH); ^{13}C nmr($CDCl_3$): δ12.3, 13.4, 55.4, 61.0(CH_3); 36.0, 38.1, 42.6, 115.6(CH_2); 112.1, 124.6, 136.4(CH); 128.6, 131.8, 144.3, 150.5(C); 167.1(C=O); Analysis: did not give satisfactory data.

Initially this reaction failed to give the allyl benzamide. In addition to recovered starting material

(>90%), the by-products 63, 64, and 65 (vide supra) were isolated (<5%). Compound 63 has the following physical properties. tlc: R_f 0.34 (Skellysolve B-ethyl acetate-acetic acid, 65:35:1, 2x); hreims: m/z calcd. for $C_{12}H_{17}NO_3(M^+)$: 223.1208; found 223.1205(78), 151(100), 150(40), 122(52), 89(22), 72(53), 71(73), 58(22).

Compound 12 has the following physical properties. tlc: R_f 0.30 (chloroform-ethyl acetate, 50:50); ir($CHCl_3$): 3600-3100(b), 1599 cm^{-1}; hreims: m/z calcd. for $C_{13}H_{19}NO_4(M^+)$: 253.1314; found 253.1319(37), 180(100), 165(41).

Compounds 63 and 64 were not formed in reactions which successfully produced the desired allyl benzamide 56. However, bromide 65 was always formed, at least in small quantities. When both compounds 56 and 65 were present in the crude reaction products, a pure sample of 65 was obtained by flash chromatography (sample dissolved in chloroform; Skellysolve B-ethyl acetate-acetic acid, 85:15:1). Compound 65 has the following physical characteristics. tlc: R_f 0.43 (Skellysolve B-ethyl acetate-acetic acid, 75:25:1, 3x); ftir($CHCl_3$ cast): 1640 cm^{-1}; hreims: m/z calcd. for $C_{13}H_{18}NO_3{}^{81}Br(M+2)$: 317.0450; found 317.0448(36); calcd. for $C_{13}H_{18}NO_3{}^{79}Br(M^+)$: 315.0470; found 315.0465(37), 286(25), 284(25), 244(100), 236(68); ^1H nmr($CDCl_3$): δ1.10(3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 1.27(3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 3.15(2H, m, CH_2), 3.6(2H, m, CH_2), 3.85(3H, s, OCH_3), 3.86(3H, s, OCH_3), 6.81(1H, d, J=8.5 Hz, ArH),

7.25(1H, d, J=8.5 Hz, ArH).

Hydrolysis and partial demethylation of compound 56

Allylbenzamide 56 (0.040 g, 1.44×10^{-4} mol) was dissolved in 6 N hydrochloric acid (15 mL) and the magnetically stirred solution was refluxed for 48 hours. Upon cooling to room temperature saturated aqueous ammonium chloride (10 mL) was added and the mixture was extracted with methylene chloride (3x 10 mL). The combined extracts were washed with water (3x 10 mL), dried over sodium sulphate, filtered, and evaporated to dryness in vacuo to give crude products (0.026 g). The crude was subjected to flash chromatography (1 cm column; sample dissolved in chloroform; Skellysolve B-ethyl acetate-acetic acid, 80:20:1) to afford a 5:1 mixture (as judged by ^{1}H nmr) of 12 and 69 (0.016 g, 55%) and a 1:1 mixture of 70 and 71 (0.004 g, 20%). The mixture of 12 and 69 has the following physical properties. tlc: R_f 0.28 and 0.45, respectively (chloroform-ethyl acetate-acetic acid, 97:2:1); ir($CHCl_3$): 3545, 3500-2400(b), 1672, 1501, 1455, 1388, 1377, 1362, 1308, 1275-1195(b), 1170, 1126, 1057, 881 cm^{-1}; ^{1}H nmr($CDCl_3$): δ1.52(d, J=6 Hz, CH_3), 2.87(d, J=7 Hz, CH_2), 3.89(s, OCH_3), 4.73(m, CH), 5.60(bs, ArOH), 6.61(d, J= 7.5 Hz, ArH), 6.65(d, J=8 Hz, ArH), 7.02(d, J=8 Hz, ArH), 7.08(d, J=7.5 Hz, ArH), 11.05(bs, peri ArOH), 11.24(bs, peri ArOH).

The mixture of 70 and 71 has the following physical properties. tlc: R_f 0.27 (Skellysolve B-ethyl acetate-acetic acid, 65:35:1); ir($CHCl_3$): 3550, 3450, 3400-2400(b), 1735, 1509, 1461, 1339, 1285-1190(b), 1162, 1108, 1082, 1052, 961 cm^{-1}; 1H nmr($CDCl_3$): δ1.00(t, J=7.5 Hz, CH_3), 1.85 and 2.07(m, CH_2), 3.94(s, OCH_3), 5.45(dd, J=6.5, 5 Hz, CH), 6.84(d, J=8 Hz, ArH), 6.87(d, J=8 Hz, ArH), 7.19(d, J=8 Hz), 7.24(d, J=8 Hz, ArH).

3-methyl-3,4-dihydro-7,8-dihydroxyisocoumarin (12, 7-hydroxymellein) and 3-ethyl-6,7-dihydroxyphthalide (70, 6-hydroxyisoochracin)

Distilled allylbenzamide 56 (1.236 g, 4.46×10^{-3} mol) was dissolved in 6 N hydrochloric acid (100 mL) and the magnetically stirred solution was refluxed for 48 hours. Upon cooling to room temperature, saturated aqueous ammonium chloride (100 mL) was added and the products were isolated by extraction with ethyl acetate (3x 100 mL). The combined ethyl acetate extracts were washed with water (1x 100 mL), dried over sodium sulphate, filtered, and evaporated to dryness in vacuo to afford a mixture (0.869 g) of mono- and diphenolic lactones (vide supra). This material was dissolved in dry methylene chloride (40 mL) and the solution was treated with boron tribromide in methylene chloride (11.4 mL, 1 g/5 mL). The solution was magnetically stirred overnight at room temperature. Water (10 mL) was added and stirring was continued for 30 minutes. Saturated aqueous

ammonium chloride (30 mL) was added and the products were extracted with chloroform (3x 40 mL). The combined organic extracts were washed with water (2x 100 mL), dried over sodium sulphate, filtered, and evaporated to dryness *in vacuo* to yield the crude lactones (0.776 g). Flash chromatography (4.5 cm column; sample dissolved in chloroform; chloroform-ethyl acetate-acetic acid, 97:2:1) afforded dihydroisocoumarin 12 (0.536 g, 62%) and phthalide 70 (0.146 g, 17%). Compound 12 has the following physical properties. mp 127.5-128.5°C ($CHCl_3$); tlc: R_f 0.58 (chloroform-methanol-acetic acid, 94:5:1); ftir($CHCl_3$ cast): 3580-2400(b), 1667, 1454, 1269, 1125 cm^{-1}; uv(CH_3OH): 229(ϵ6400), 261(ϵ5800), 334(ϵ3700)nm; hreims: m/z calcd. for $C_{10}H_{10}O_4(M^+)$: 194.0579; found 194.0582(100), 176(24), 165(20), 148(54); ^1H nmr($CDCl_3$): δ1.54(3H, d, J=7 Hz, CH_3), 2.90(2H, d, J=7 Hz, CH_2), 4.77(1H, sextet, J=7 Hz, CH), 5.66(1H, bs, ArOH), 6.65(1H, d, J=8 Hz, ArH), 7.12(1H, d, J=8 Hz, ArH), 11.11(1H, bs, *peri* ArOH); ^{13}C nmr(CD_3OD): δ20.8(CH_3); 34.7(CH_2); 78.5, 109.3, 118.7(CH); 122.6, 131.1, 145.6, 151.3(C); 171.9(C=O); Analysis: calcd. for $C_{10}H_{10}O_4$: C 61.85, H 5.19; found C 61.70, H 5.07.

Compound 70 has the following properties. mp 160-161°C (CH_2Cl_2-acetone); tlc: R_f 0.36 (chloroform-methanol-acetic acid, 94:5:1); ftir(CH_3OH cast): 3580-2400(b), 1724, 1519, 1282 cm^{-1}; uv(CH_3OH): 228(ϵ6200), 322(ϵ3550)nm; hreims: m/z calcd. for $C_{10}H_{10}O_4(M^+)$: 194.0579; found 194.0571(67), 176(27), 165(100), 148(29), 137(31); ^1H nmr(CD_3OD):

δ0.91(3H, t, J=7 Hz, CH_3), 1.71(1H, ddq, J=14, 7, 4 Hz) and 2.02(1H, ddq, 17, 7, 6.5 Hz, CH_2), 5.34(1H, dd, J=6.5, 4 Hz, CH), 6.76(1H, d, J=8 Hz, ArH), 7.10(1H, d, J=8 Hz, ArH); ^{13}C nmr(CD_3OD): δ8.8(CH_3); 28.8(CH_2); 83.4, 113.4, 123.3(CH); 116.3, 142.5, 145.7, 146.5(C); 172.2(C=O); Analysis: calcd. for $C_{10}H_{10}O_4$: C 61.85, H 5.19; found C 62.01, H 5.24.

N,N-diethyl-2,3-dimethoxy-6-(2-hydroxypropyl)benzamide (76, oxymercuration-demercuration route)

Tetrahydrofuran (1 mL) was added to a magnetically stirred solution of mercuric acetate (0.062 g, 1.95×10^{-4} mol) dissolved in water (3 mL), resulting in formation of a powdery yellow precipitate. A solution of allylbenzamide 56 (0.049 g, 1.77×10^{-4} mol) dissolved in tetrahydrofuran (3 mL) was added, causing the precipitate to disappear. The reaction was stirred for two hours, then 3 M sodium hydroxide (1 mL) and freshly prepared 0.5 M sodium borohydride in 3 M sodium hydroxide (1 mL) were added sequentially, the latter resulting in the precipitation of metallic mercury. The reaction was diluted with brine (10 mL) and extracted with chloroform (3x 10 mL). The combined organic layers were dried over sodium sulphate, filtered, and evaporated to dryness in vacuo. The crude material (0.066 g) was subjected to flash chromatography (1 cm; chloroform-ethyl acetate, 88:12 to elute starting material 56 (2 mg, 4%), then 75:25 to elute 83 (2 mg, 4%), then 50:50 to elute 76 (26 mg, 50%), and chloroform-methanol, 95:5 to

elute 84 (7 mg, 13%). Compound 83 exists as a pair of rotational isomers (1:1) and has the following physical properties. tlc: R_f 0.38 (chloroform-ethyl acetate, 50:50). ftir($CHCl_3$ cast): 3600-3100(b), 2930, 1612, 1485, 1275, 1060 cm^{-1}. hreims: no M^+; m/z calcd. for $C_{16}H_{23}NO_3$(M^+-H_2O): 277.1678; found 277.1675(18), 251(43), 236(97), 220(32), 205(56), 179(73). cims: 296(M+1). ^1H nmr($CDCl_3$): δ1.00(3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 1.02(3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 1.27(3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 1.28(3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 1.77(1H, dd, J=12, 3.5 Hz), 2.01(1H, dd, J=12, 5.5 Hz), 2.06(1H, dd, J=11.5, 7.25 Hz), and 2.18(1H, dd, J=11.5, 5.25 Hz, 4x homobenzylic H), 2.40(1H, dd, J=13.25, 8.5 Hz), 2.68(1H, dd, J=13.5, 5.5 Hz), 2.75(1H, dd, J=13.5, 4 Hz), and 2.84(1H, dd, J=13.25, 3.5 Hz, 4x benzylic H), 3.06-3.3(4H, m) and 3.48(2H, m, 3x $\underline{CH_2}CH_3$), 3.83(3H, s, OCH_3), 3.85(3H, s, OCH_3), 3.88(3H, s, OCH_3), 3.89(3H, s, OCH_3), 3.7-4.0(4H, m, $\underline{CH_2}CH_3$ and $\underline{CH_2}OH$), 4.30(1H, m) and 4.62(1H, m, $\underline{CH_2}OH$), 6.95(1H, d, J=8 Hz, ArH), 6.99(2H, s, 2x ArH), 7.03(1H, d, J=8 Hz, ArH).

Compound 76 exists as a pair of rotational isomers (1:1) and has the following physical properties. bp 172-184°C/0.07-0.08 Torr. tlc: R_f 0.24 and 0.17 (chloroform-ethyl acetate, 50:50). ftir($CHCl_3$ cast): 3600-3100(b), 2970, 2935, 1615, 1487, 1272, 1060 cm^{-1}. hreims: m/z calcd. for $C_{16}H_{25}NO_4$(M^+): 295.1784; found 295.1789(0.4), 251(44), 236(100), 220(41), 179(90), 178(20).

cims: 296(M+1). ^{1}H nmr($CDCl_3$): δ1.03(6H, t, J=7 Hz, 2x $CH_2\underline{CH_3}$), 1.21(3H, d, J=6 Hz, $CH(OH)\underline{CH_3}$), 1.27(3H, d, J=6 Hz, $CH(OH)\underline{CH_3}$), 1.27(3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 1.28(3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 1.7(1H, bs, OH), 2.35(1H, dd, J=14, 9.5 Hz), 2.54(1H, dd, J=14, 6.5 Hz), 2.70(1H, dd, J=14, 10.5 Hz), and 2.72(1H, dd, J=14, 9.5 Hz, 4x $ArCH_2$), 3.05(1H, bs, OH), 3.09-3.29(4H, m, 2x $\underline{CH_2}CH_3$), 3.44-3.8(4H, m, 2x $\underline{CH_2}CH_3$), 3.82(3H, s, OCH_3), 3.84(3H, s, OCH_3), 3.87(6H, s, 2x OCH_3), 4.12(2H, m, 2x $\underline{CH}OH$), 6.90(1H, d, J=8 Hz, ArH), 6.94(1H, d, J=8 Hz, ArH), 7.01(1H, d, J=8 Hz, ArH), 7.015(1H, d, J=8 Hz, ArH). ^{13}C nmr($CDCl_3$): δ12.4, 13.5, 13.7, 18.6, 22.6, 24.3, 55.6(2x), 61.2(2x)(CH_3); 38.8, 38.9, 41.1, 42.1, 42.8, 43.0(CH_2); 66.6, 68.9, 112.1, 112.9, 125.5, 126.2(CH); 127.2, 129.0, 131.7, 132.2, 144.0, 144.5, 150.5, 150.8(C); 168.0, 168.5(C=O).

Compound 84 exists as a pair of rotational isomers (1:1) and has the following physical properties. tlc: R_f 0.06 (chloroform-ethyl acetate, 50:50). ftir($CHCl_3$ cast): 3600-3100(b), 2935, 1605, 1485, 1272, 1061 cm^{-1}. hreims: no M^+; m/z calcd. for $C_{16}H_{23}NO_4$(M^+-H_2O): 293.1627; found 293.1625(0.6), 280(21), 251(60), 236(100), 220(39), 179(94). cims: m/z 312(M+1). ^{1}H nmr($CDCl_3$): δ1.03(3H, t, J=6.75 Hz, $CH_2\underline{CH_3}$), 1.035(3H, t, J=6.75 Hz, $CH_2\underline{CH_3}$), 1.29(3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 1.295(3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 2.48(1H, dd, J=14, 9.5 Hz) and 2.52(1H, dd, J=14, 9 Hz, $ArCH_2$), 2.58(1H, bs, OH), 2.75(1H, dd, J=13.5, 3.5 Hz) and 2.83(1H, dd, J=13.5, 5.25 Hz, $ArCH_2$), 3.02(1H, bs, OH), 3.05-3.30(4H, m,

2x $\underline{CH_2}CH_3$), 3.42-3.56(5H, m, 2x $\underline{CH_2}CH_3$ and OH), 3.64-3.9(6H, m, 2x $\underline{CH_2}OH$ and 2x $\underline{CH}(OH)$), 3.83(3H, s, OCH_3), 3.84(3H, s, OCH_3), 3.88(3H, s, OCH_3), 3.89(3H, s, OCH_3), 4.82(1H, bd, J=4.5 Hz, OH), 6.92(1H, d, J=8 Hz, ArH), 6.96(1H, d, J=8 Hz, ArH), 6.98(1H, d, J=8 Hz, ArH), 7.06(1H, d, J=8 Hz, ArH).

Acetylation of alcohol 76 derived from oxymercuration-demercuration

Alcohol 76 (5.6 mg) was dissolved in acetic anhydride (0.5 ml) and pyridine (0.5 ml) and the solution was magnetically stirred for 1.5 hours at room temperature. Toluene was added and the solvents were removed in vacuo. Flash chromatography (1 cm column, Skellysolve B-ethyl acetate, 50:50) afforded acetate 79 (3.8 mg).

N,N-diethyl-2,3-dimethoxy-6(2,3-diacetoxypropyl)benzamide (85)

Diol 84 (4 mg) was dissolved in acetic anhydride (0.5 mL) and pyridine (0.5 mL) and the solution was magnetically stirred overnight at room temperature. Toluene was added and the solvents were removed in vacuo. The crude product (4 mg) was subjected to flash chromatography (0.5 cm column, chloroform-ethyl acetate, 75:25) to yield diacetate 85 (2.5 mg), which has the following physical properties. tlc: R_f 0.60 (chloroform-methanol, 95:5); ftir($CHCl_3$ cast): 2930, 1742, 1630, 1224, 1057 cm^{-1}; hreims: m/z calcd. for $C_{20}H_{29}NO_7(M^+)$: 395.1944; found 395.1948(24), 335(100),

292(28), 276(81), 262(19), 236(28), 221(26), 220(54), 205(24), 203(53), 191(29), 179(40), 162(37), 72(40); ^{1}H nmr($CDCl_3$): δ1.06(3H, t, J=6.75 Hz, $CH_2\underline{CH_3}$), 1.09(3H, t, J=6.75 Hz, $CH_2\underline{CH_3}$), 1.26(3H, t, J=6.75 Hz, $CH_2\underline{CH_3}$), 1.27(3H, t, J=6.75 Hz, $CH_2\underline{CH_3}$), 2.02(3H, s, $COCH_3$), 2.03(3H, s, $COCH_3$), 2.07(3H, s, $COCH_3$), 2.08(3H, s, $COCH_3$), 2.69-2.91(4H, m, 2x $ArCH_2$), 3.09-3.21(4H, m, 2x $\underline{CH_2}CH_3$), 3.49-3.77(4H, m, 2x $\underline{CH_2}CH_3$), 3.83(3H, s, OCH_3), 3.84(3H, s, OCH_3), 3.885(3H, s, OCH_3), 3.89(3H, s, OCH_3), 4.00(1H, dd, J=11.5, 6.0 Hz) and 4.06(1H, dd, J=11.5, 5.5 Hz, CH_2(OAc)), 4.28(1H, dd, J=11.75, 3.5 Hz), and 4.32(1H, dd, J=11.75, 2.75 Hz, CH_2(OAc)), 5.29(1H, m, CH(OAc)), 5.36(1H, m, CH(OAc)), 6.87(1H, d, J=8 Hz, ArH), 6.90(1H, d, J=8 Hz, ArH), 6.98(1H, d, J=8 Hz, ArH), 7.03(1H, d, J=8 Hz, ArH).

N,N-diethyl-2,3-dimethoxy-6-(2-hydroxypropyl)benzamide (76, ortho lithiation route)

A representative example is given of a typical run. Dimethoxybenzamide 62 (3.269 g, 1.38×10^{-2} mol) was dissolved in dry tetrahydrofuran (100 mL) under an argon atmosphere, and the magnetically stirred solution was cooled to -78°C in a dry-ice bath. TMEDA (3.1 mL, 2.07×10^{-2} mol) and s-butyllithium (1.26 M in cyclohexane, 16.4 mL, 2.07×10^{-2} mol) were added and the solution was stirred for 30 minutes. Freshly prepared magnesium bromide etherate[72] (2.62 N in ether, 15.7 mL, 4.14×10^{-2} mol) was added and stirring was continued for 40 minutes. After the addition of propylene

oxide (2.9 mL, 4.14×10^{-2} mol) the reaction was allowed to warm to room temperature overnight. Saturated aqueous ammonium chloride (100 mL) was poured into the reaction mixture and the products were extracted with ethyl acetate (3x 100 mL). The combined organic layers were dried over sodium sulphate, filtered, and evaporated *in vacuo* to afford crude material (4.955 g). The crude material was subjected to flash chromatography (4.5 cm column) to give recovered starting material (62, 0.233 g, 7%) by elution with chloroform-ethyl acetate, 80:20, and alcohol 76 (3.493 g (2.995 g, 73%, *vide infra*)) containing traces of less polar impurities by tlc) by elution with ethyl acetate. The best reproducible yields of 76 ranged from 66 to 73%.

N,N-diethyl-2,3-dimethoxy-6-(2-acetoxypropyl)benzamide (79)

Crude alcohol 76 (3.324 g, containing slightly less polar impurities by tlc) was dissolved in pyridine (10 mL) and acetic anhydride (10 mL). The magnetically stirred solution was refluxed for two hours, then allowed to cool to room temperature and diluted with ice water (20 mL). The aqueous phase was extracted with ethyl acetate (3x 20 mL) and the combined organic layers were dried over sodium sulphate, filtered, and evaporated *in vacuo* to afford crude material (4.303 g). The crude material was subjected to flash chromatography (4.5 cm column) to yield a mixture (0.112 g) containing phthalides 88 and 89 (*vide infra*) by elution with Skellysolve B-ethyl acetate, 75:25 and acetate

79 (3.414 g) by elution with Skellysolve B-ethyl acetate, 50:50. Compound 79 has the following physical properties. tlc: R_f 0.57 (chloroform-ethyl acetate, 50:50); ftir($CHCl_3$ cast): 2985, 2935, 1735, 1635, 1245, 1060 cm^{-1}; hreims: m/z calcd. for $C_{18}H_{27}NO_5(M^+)$: 337.1889; found 337.1893(20), 277(41), 236(18), 223(21), 205(100); ^1H nmr($CDCl_3$): δ1.06(3H, t, J=6.75 Hz, $CH_2\underline{CH_3}$), 1.09(3H, t, J=6.75 Hz, $CH_2\underline{CH_3}$), 1.222(3H, d, J=6 Hz, CH(OAc)$\underline{CH_3}$), 1.227(3H, d, J=6 Hz, CH(OAc)$\underline{CH_3}$), 1.26(3H, t, J=6.75 Hz, $CH_2\underline{CH_3}$), 1.27(3H, t, J=6.75 Hz, $CH_2\underline{CH_3}$), 2.00(3H, s, $COCH_3$), 2.01(3H, s, $COCH_3$), 2.59(1H, dd, J=14.5, 6.5 Hz), 2.75(1H, d, J=5.5 Hz), 2.755(1H, d, J=6.5 Hz), and 2.84(1H, dd, J=14.5, 7 Hz, 2x $ArCH_2$), 3.13(4H, m, 2x $\underline{CH_2}CH_3$), 3.44-3.8(4H, m, 2x $\underline{CH_2}CH_3$), 3.83(3H, s, OCH_3), 3.84(3H, s, OCH_3), 3.89(3H, s, OCH_3), 3.90(3H, s, OCH_3), 5.08(1H, sextet, J=6.5 Hz, CHOAc), 5.16(1H, sextet, J=6.5 Hz, CHOAc), 6.85(1H, d, J=8 Hz, ArH), 6.87(1H, d, J=8 Hz, ArH), 6.96(1H, d, J=8 Hz, ArH), 7.00(1H, d, J=8 Hz, ArH); ^{13}C nmr($CDCl_3$): δ12.3, 13.3, 13.4, 19.4, 19.8(2x), 21.0(2x), 55.5(2x), 61.1(2x)(CH_3); 37.8, 37.9, 38.2, 38.3, 42.6(2x)(CH_2); 70.3, 70.7, 111.8, 112.0, 125.0, 125.8(CH); 126.4, 126.6, 132.4, 132.5, 144.4, 144.5, 150.8(2x)(C); 167.0, 167.1, 170.0(2x)(C=O).

An early run of the acetylation reaction (performed on 0.587 g of crude alcohol 76) yielded two additional by-products, phenolic acetate 86 and diphenyl 87. The reaction was carried out at room temperature for 101 hours, then toluene was added and the solvents were removed in

vacuo. Flash chromatography (4 cm column) yielded acetate 79 (0.466 g) and 86 (0.044 g, 2% from 62), by elution with Skellysolve B-ethyl acetate, 50:50, and mixed fractions (0.013 g) containing 87 by elution with ethyl acetate. Compound 87 was further purified by ptlc (10x20 cm, chloroform-methanol, 98:2, 2x), then flash chromatography (1 cm column, chloroform methanol, 98:2) to afford 87 (9 mg, 0.2% from 62).

Compound 86 has the following physical properties. mp 77-79°C (Skellysolve B-ether); tlc: R_f 0.45 (chloroform-ethyl acetate, 50:50); ftir($CHCl_3$ cast): 2970, 2935, 1769, 1638, 1482, 1205, 1060 cm^{-1}; hreims: m/z calcd. for $C_{15}H_{21}NO_5(M^+)$: 295.1420; found 295.1423(8), 253(50), 180(100), 165(20), 72(17); ^1H nmr($CDCl_3$): δ1.08(3H, t, J=6.75 Hz, $CH_2\underline{CH_3}$), 1.24(3H, t, J=6.75 Hz, $CH_2\underline{CH_3}$), 2.23(3H, s, $COCH_3$), 3.16(2H, m, $\underline{CH_2}CH_3$), 3.41(1H, m) and 3.76(1H, m, $\underline{CH_2}CH_3$), 3.88(3H, s, OCH_3), 3.90(3H, s, OCH_3), 6.90(1H, d, J=8.5 Hz, ArH), 6.94(1H, d, J=8.5 Hz, ArH).

Compound 87 possesses the following physical characteristics. tlc: R_f 0.55 (chloroform-methanol, 95:5); ftir($CHCl_3$ cast): 2968, 2935, 1628, 1460, 1427, 1293, 1255, 1058 cm^{-1}; hreims: m/z calcd. for $C_{25}H_{34}N_2O_5(M^+)$: 442.2468; found 442.2461(36), 411(28), 271(61), 270(30), 100(74), 72(100);cims: m/z 443(M+1); ^1H nmr($CDCl_3$): δ0.80(3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 0.94(3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 1.01(3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 1.19(3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 2.87-3.13(4H, m, 2x $\underline{CH_2}CH_3$), 3.28(1H, m) and 3.45-3.67(3H, m, 2x $\underline{CH_2}CH_3$),

3.71(3H, s, OCH_3), 3.78(3H, s, OCH_3), 3.93(3H, s, OCH_3), 6.90(1H, dd, J=7.5, 1 Hz, ArH), 6.96(1H, dd, J=7.5, 1 Hz, ArH), 7.00(1H, d, J=8 Hz, ArH), 7.30(1H, d, J=8 Hz, ArH), 7.34(1H, dd, J=7.5, 7.5 Hz, ArH).

Hydrolysis of acetate 79

To a magnetically stirred solution of acetate 79 (3.414 g, 1.01×10^{-2} mol) in methanol (50 mL) was added anhydrous potassium carbonate (1.40 g, 1.01×10^{-2} mol). The reaction was stirred for 48 hours at room temperature. The reaction mixture was neutralised with 5% HCl (v/v) and diluted with brine (50 mL). The product was extracted with ethyl acetate (3x 50 mL). The combined extracts were dried over sodium sulphate, filtered, and evaporated *in vacuo* to afford alcohol 76 (2.995 g, quantitative). This material has physical properties identical with those of compound 76 prepared *via* the oxymercuration-demercuration route (*vide supra*).

In an earlier run the acetate 79 contained small amounts of impurities (apparently carried over from the *ortho* lithiation reaction used to generate 76) which were separated following the hydrolysis. Thus flash chromatography (4 cm column) of the crude hydrolysis products (2.286 g) yielded a 3.5:1 (determined by ^1H nmr peak intrgrations) mixture of phthalides 88 and 89 (0.067 g) and benzamide 62 (0.016 g) by elution with chloroform-ethyl acetate, 95:5, and alcohol 76 (2.192 g) by elution with

ethyl acetate. The mixture of 88 and 89 has the following physical properties. tlc: R_f 0.45 (chloroform-ethyl acetate, 95:5); ftir($CHCl_3$ cast): 2970, 2935, 2845, 1742, 1501, 1301, 1277, 1259, 1091, 1045, 1030, 972, 932, 828 cm^{-1}; hreims: m/z calcd. for $C_{12}H_{14}O_4(M^+)$: 222.0892; found 222.0893(54), 207(100), 193(36); ^1H nmr($CDCl_3$): δ0.84(t, J=7.5 Hz, $CH_2\underline{CH_3}$), 1.52(s, gem dimethyls), 1.66(m) and 1.96(m, CH_2), 3.81(s, OCH_3), 4.00(s, OCH_3), 5.22(m, CH), 6.94(d, J=8 Hz, ArH), 6.98(d, J=8 Hz, ArH), 7.15(d, J=8 Hz, ArH), 7.16(d, J=8 Hz, ArH).

^{13}C nmr($CDCl_3$): δ8.5, 27.5(2x), 56.8(2x), 62.0(2x)(CH_3); 27.8(CH_2); 80.7, 115.0(2x), 115.9, 116.1(CH); 83.6, 117.3(2x), 142.7, 148.0, 148.2, 152.2(2x), 152.3(C); 166.9, 167.9(C=O).

Interconversion of phenol 64 and phenolic acetate 86

A solution of phenol 64 (20 mg) in pyridine (1.0 mL) and acetic anhydride (1.0 mL) was magnetically stirred overnight at room temperature. Toluene was added and the solvents were removed in vacuo. Flash chromatography (1 cm column, chloroform-ethyl acetate, 50:50) of the crude reaction products afforded the acetate 86 (19 mg, 83%). This sample of 86 was identical (tlc and ftir) with that isolated from the acetylation reaction of crude 76 (vide supra).

Potassium carbonate (2 mg) was added to a magnetically stirred solution of acetate 86 (1.6 mg) in methanol (0.5

mL). After 30 minutes at room temperature only a spot corresponding to phenol 12 was evident by tlc (chloroform-ethyl acetate, 50:50, 2x).

N,N-diethyl-2,3-dimethoxy-6-(2-propanoyl)benzamide (77, Jones oxidation route)

A magnetically stirred solution of alcohol 76 (0.403 g, 1.37×10^{-3} mol) in acetone (15 mL) was cooled in an ice bath (0°C). The reaction was monitored by tlc as Jones reagent[81] was added drop-wise. The starting material was consumed after the addition of 25 drops, however, in order to try to consume a by-product which is slightly more polar than the desired compound (77), the reaction was allowed to warm to room temperature and a further 20 drops of reagent were added. After stirring overnight the by-product was still present. The acetone layer was decanted off and the aqueous phase was extracted with hot ether (3x 20 mL). The combined organic layers were washed with saturated aqueous sodium bicarbonate (1x 20 mL), brine (1x 20 mL), dried over sodium sulphate, and evaporated in vacuo to afford crude reaction products (0.286 g). Flash chromatography (3 cm column, chloroform-ethyl acetate, 60:40) yielded ketone 77 (0.163 g, 41%) and impure by-product (0.020 g). The latter was further purified by ptlc (20x20 cm, chloroform-methanol, 98:2, 3x) to give aldehyde 99 (7 mg, 2%). Ketone 77 has the following physical properties. bp 175-179°C/0.2-0.25 Torr; tlc: R_f 0.42 (chloroform-ethyl acetate, 50:50); ftir($CHCl_3$

cast): 1715, 1630, 1490, 1270, 1061 cm^{-1}; uv(methanol): 234(ϵ5200), 282(ϵ2300)nm; hreims: m/z calcd. for $C_{16}H_{23}NO_4(M^+)$: 293.1627; found 293.1624(11), 251(20), 236(100), 221(40), 220(95), 179(76), 178(23); ^1H nmr($CDCl_3$): δ1.05(3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 1.24(3H, t, J=6.75 Hz, $CH_2\underline{CH_3}$), 2.18(3H, s, $COCH_3$), 3.04-3.24(2H, m, $\underline{CH_2}CH_3$), 3.46-3.7(2H, m, $\underline{CH_2}CH_3$), 3.56(1H, d, J=16 Hz) and 3.70(1H, d, J=16 Hz, $ArCH_2$), 3.83(3H, s, OCH_3), 3.88(3H, s, OCH_3), 6.91(1H, d, J=8 Hz), 6.94(1H, d, J=8 Hz); ^{13}C nmr($CDCl_3$): δ12.4, 13.3, 29.2, 55.5, 61.1(CH_3); 38.2, 42.6, 46.6(CH_2); 112.2, 126.2(CH); 123.5, 132.3, 144.5, 151.2(C); 167.0, 205.7(C=O); Analysis: calcd. for $C_{16}H_{23}NO_4$: C 65.51, H 7.90, N 4.77; found C 65.39, H 7.77, N 4.75.

Aldehyde 99 has the following physical properties. tlc: R_f 0.34 (chloroform-ethyl acetate, 50:50); ftir($CHCl_3$ cast): 2975, 2935, 2820, 2722, 1693, 1636, 1587, 1569, 1279, 1055 cm^{-1}; hreims: m/z calcd. for $C_{14}H_{19}NO_4(M^+)$: 265.1314; found 265.1307(11), 236(100), 193(68), 72(38); ^1H nmr($CDCl_3$): δ1.02(3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 1.31(3H, t, J=6.75 Hz, $CH_2\underline{CH_3}$), 3.14(2H, m, CH_2), 3.66(2H, m, CH_2), 3.89(3H, s, OCH_3), 3.99(3H, s, OCH_3), 7.05(1H, d, J=8 Hz, ArH), 7.73(1H, d, J=8 Hz, ArH), 9.87(1H, s, CHO).

Ketone 77 via oxidation with pyridinium chlorochromate

Pyridinium chlorochromate[82] (0.204 g, 9.46×10^{-4} mol) was dissolved in methylene chloride (3 mL). A solution of alcohol 76 (0.186 g, 6.31×10^{-4} mol) in methylene chloride (5

mL) was added and the solution was magnetically stirred at room temperature for 24 hours. The reaction mixture was diluted with ether (40 mL) and the solution was decanted. The solid residue was extracted with hot ether (2x 20 mL). The combined organic layers were filtered through a Florisil pad (rinsing with more hot ether), dried over sodium sulphate, filtered, and evaporated *in vacuo* to give crude products (0.170 g). The crude products were subjected to flash chromatography (2 cm column, chloroform-ethyl acetate, 60:40) to afford ketone *77* (0.137 g, 74%) and aldehyde *99* (0.006 g, 3%).

Ketone 77 via Swern[83] *oxidation*

A magnetically stirred solution of oxalyl chloride (0.875 mL, 1.00×10^{-2} mol, freshly distilled under an argon atmosphere) in dry methylene chloride (25 mL) was cooled to -78°C in a dry-ice bath under an atmosphere of argon. A solution of dimethyl sulphoxide (1.20 mL, 1.67×10^{-2} mol, distilled at reduced pressure) in dry methylene chloride (5 mL) was added over *ca.* 5 minutes. After stirring for 20 minutes, a solution of alcohol *76* (1.975 g, 6.69×10^{-3} mol) in dry methylene chloride (10 mL) was added over *ca.* 5 minutes. Stirring was continued for 15 minutes, then dry triethylamine (4.6 mL, 3.34×10^{-2} mol) was added over *ca.* 5 minutes. After a further 5 minutes the dry-ice bath was removed and the reaction was allowed to warm to room temperature. The reaction mixture was diluted with water

(30 mL) and the aqueous phase was extracted with methylene chloride (3x 30 mL). The combined organic layers were dried over sodium sulphate, filtered and evaporated *in vacuo*. The crude product was subjected to flash chromatography (4.5 cm column, chloroform-ethyl acetate, 99:1 (13 fractions) and 95:5 (fractions 14-35) to elute non-polar impurities, then 60:40) to give ketone **77** (1.840 g, 94%).

Methylation of lactone 12

To a magnetically stirred solution of lactone **12** (5.5 mg, 2.84×10^{-5} mol) in dry acetone (10 ml) was added excess methyl iodide (1.0 ml) and anhydrous potassium carbonate (5.5 mg, 3.98×10^{-5} mol). The reaction was heated at 60°C in a constant temperature oil bath for 22 hours. The acetone was evaporated *in vacuo* and the residue was redissolved in chloroform (10 ml). Saturated aqueous ammonium chloride (10 ml) was added and the aqueous phase was extracted with chloroform (3x 10 ml). The combined chloroform layers were dried over sodium sulphate, filtered and evaporated to dryness *in vacuo* to afford crude products (7 mg). Flash chromatography (1 cm column, sample dissolved in chloroform, Skellysolve B-ethyl acetate-acetic acid, 75:25:1) gave a 1:2 mixture (4 mg) of starting material (**12**) and 7-methoxylactone (**73**) and a 2:1 mixture (2 mg) of 8-methoxylactone (**74**) and 7,8-dimethoxylactone (**75**). The above ratios were determined from ^1H nmr peak integrations. Compounds **12**, **73**, **74**, and **75** have R_f values of 0.27, 0.45,

0.20, and 0.25 respectively (chloroform-ethyl acetate-acetic acid, 97:2:1). The ^1H nmr ($CDCl_3$) data of the two mixtures follow.

12+73: δ1.53(d, J= 7 Hz, $CH\underline{CH_3}$), 2.88(d, J=7 Hz, CH_2), 3.90(s, OCH_3), 4.64-4.86(m, CH), 5.56(bs, ArOH), 6.64(d, J=8 Hz, ArH), 6.66(d, J=8 Hz, ArH), 7.03(d, J=8 Hz, ArH), 7.10(d, J=8 Hz, ArH), 11.06(bs, peri ArOH), 11.25(bs, peri ArOH).

74+75: δ1.48(d, J=6 Hz, $CH\underline{CH_3}$), 1.51(d, J=6 Hz, $CH\underline{CH_3}$), 2.84(d, J=8.5 Hz, CH_2), 2.86(d, J=8.5 Hz, CH_2), 3.89(s, OCH_3), 3.97(s, OCH_3), 3.99(s, OCH_3), 4.48-4.72(m, CH), 6.00(bs, ArOH), 6.90(d, J=8.5 Hz, ArH), 6.92(d, J=8.5 Hz, ArH), 7.08(d, J=8.5 Hz, ArH), 7.16(d, J=8.5 Hz, ArH).

2-N,N-diethylbenzamido-2',3',3,4-tetramethoxybenzophenone (93)

Benzamide 62 (1.222 g, 5.16×10^{-3} mol) was dissolved in dry tetrahydrofuran (40 mL) and the magnetically stirred solution was cooled to -78°C under an argon atmosphere. TMEDA (1.95 mL, 1.29×10^{-2} mol) was added, followed by s-butyllithium (1.29 M in cyclohexane, 10.0 mL, 1.29×10^{-2} mol), and the reaction was stirred for 30 minutes. Propylene oxide (3.6 mL, 5.16×10^{-2} mol) was added, causing the reaction mixture to become deep purple in colour. Upon allowing the reaction to warm to room temperature overnight (purple colour disappeared), saturated aqueous ammonium chloride (40 mL) was added, and the aqueous phase was

extracted with ethyl acetate (3x 40 mL). The combined organic layers were dried over sodium sulphate, filtered, and evaporated *in vacuo* to afford crude material (1.551 g). Flash chromatography of the crude reaction products (4.5 cm column) yielded starting material (62, 0.341 g, 28%) by elution with chloroform-ethyl acetate, 90:10, benzophenone 93 (0.147 g) by elution with chloroform-ethyl acetate, 80:20, and a mixture of 93 and alcohol 76 (0.504 g) by elution with ethyl acetate. Acetylation (*vide supra*) of the mixture of 76 and 93 facilitated isolation of the remainder of 93 by flash chromatography (3 cm column, Skellysolve B-ethyl acetate, 50:50; overall yield 0.232 g, 11%). Acetate 79 (0.409 g, representing 0.358 g of 76, 24%) was eluted with ethyl acetate. Compound 93 has the following physical properties. tlc: R_f 0.35 (chloroform-ethyl acetate, 50:50); ftir($CHCl_3$ cast): 2970, 2930, 1665, 1636, 1477, 1274 cm^{-1}; hreims: m/z calcd. for $C_{22}H_{27}NO_6(M^+)$: 401.1838; found 401.1848(7), 329(75), 315(20), 299(20), 72(100); ^1H nmr($CDCl_3$): δ1.07(3H, t, J=6.75 Hz, $CH_2\underline{CH_3}$), 1.30(3H, t, J=6.75 Hz, $CH_2\underline{CH_3}$), 3.12-3.30(2H, m, CH_2), 3.54-3.75(2H, m, CH_2), 3.73(3H, s, OCH_3), 3.88(3H, s, OCH_3), 3.89(3H, s, OCH_3), 3.91(3H, s, OCH_3), 6.84(1H, d, J=8 Hz, ArH), 6.93(1H, dd, J=7.5, 1 Hz, ArH), 7.04(1H, dd, J=7.5, 1 Hz, ArH), 7.11(1H, dd, J=7.5 Hz, ArH), 7.27(1H, d, J=8 Hz, ArH); ^{13}C nmr($CDCl_3$): δ12.0, 13.2, 55.7(2x), 61.5(2x)(CH_3); 38.4, 42.6(CH_2); 110.3, 114.0, 120.3, 123.7, 130.2(CH); 127.8, 133.2, 134.3, 145.1, 146.5, 152.4, 156.4(C); 166.7,

193.7(C=O).

2,3,3',4'-tetrahydroxybenzophenone (96)

A magnetically stirred solution of benzophenone 93 (0.054 g, 1.35×10^{-4} mol) in 6 N hydrochloric acid (10 mL) was refluxed for 48 hours. After cooling, the reaction mixture was diluted with saturated aqueous ammonium chloride (10 mL) and extracted with ethyl acetate (3x 20 mL). The combined extracts were washed with water (1x 50 mL), dried over sodium sulphate, filtered, and evaporated to dryness *in vacuo*. The crude products (0.041 g) were dissolved in dry methylene chloride (10 mL) and treated with a solution of boron tribromide in methylene chloride (1 g/5 mL, 0.43 mL). The reaction mixture was magnetically stirred overnight, then water (5 mL) was added, and stirring was continued for 30 minutes. Saturated aqueous ammonium chloride (10 mL) was added and the aqueous phase was extracted with ethyl acetate (3x 10 mL). The combined organic layers were washed with water (1x 50 mL), dried over sodium sulphate, filtered, and evaporated to dryness *in vacuo*. Dry flash chromatography[88] (1 cm column) of the crude (0.050 g) yielded a mixture (11 mg) of an unidentified compound plus 94 from elution with chloroform (fractions 1-4) and chloroform-methanol, 99.5:0.5 (fractions 5-9), 95 (4 mg, 11%, fractions 23-30) and 96 (3 mg, 9%, fractions 42-49) by elution with chloroform-methanol, 99:1. Impure 94 was further subjected to flash chromatography (1 cm column, chloroform-ethyl

acetate, 92.5:7.5) to give 94 (1.2 mg). Compound 94 has the following physical properties. tlc: R_f 0.57 (chloroform-methanol, 90:10); ^{1}H nmr($CDCl_3$): δ4.00(3H, s, OCH_3), 5.75(2H, bs, 2x ArOH), 6.81(1H, dd, J=8, 8 Hz, H-5), 6.95(1H, d, J=8 Hz, H-5'), 7.16(1H, dd, J=8, 1 Hz, H-4*), 7.22(1H, dd, J=8, 1 Hz, H-6*), 7.32(1H, dd, J=8, 2 Hz, H-6'), 7.34(1H, d, J=2 Hz, H-2'), 12.16(1H, bs, peri ArOH); (CD_3COCD_3+D_2O): δ3.94(3H, s, OCH_3), 6.82(1H, dd, J=8, 8 Hz, H-5), 7.09(1H, d, J=8.5 Hz, H-5'), 7.105 (1H, dd, J=8, 1.5 Hz, H-4*), 7.15 (1H, dd, J=8, 1.5 Hz, H-6*), 7.256 (1H, d, J=2 Hz, H-2'), 7.261(1H, dd, J=8.5, 2 Hz, H-6'); (*assignments interchangeable).

Compound 95 has the following physical properties. tlc: R_f 0.41 (chloroform-methanol, 90:10); ^{1}H nmr($CDCl_3$): δ3.91(3H, s, OCH_3), 6.83(1H, dd, J=8, 8 Hz, H-5), 6.94(1H, d, J=8 Hz, H-5'), 7.09(1H, dd, J=8, 1 Hz, H-4*), 7.22(1H, dd, J=8, 2 Hz, H-6'), 7.24(1H, dd, J=8, 1 Hz, H-6*), 7.29(1H, d, J=2 Hz, H-2'), 11.9(1H, bs, peri ArOH); (CD_3COCD_3+D_2O): δ3.88(3H, s, OCH_3), 6.88(1H, dd, J= 7.75, 7.75 Hz, H-5), 6.94(1H, d, J=8 Hz, H-5'), 7.17(2H, d, J=7.75 Hz, H-4* and H-6*), 7.19(1H, d, J=8 Hz, H-6'*), 7.30(1H, s, H-2'); (*assignments interchangeable).

Compound 96 has the following physical properties. mp 198-201°C (benzene-ethyl acetate); tlc: R_f 0.33 (chloroform-methanol, 90:10); ftir(CH_3OH cast): 3620-2360(b), 1625, 1591, 1447, 1315, 1265, 1225, 1116, 851, 772, 753 cm^{-1}; hreims: m/z calcd. for $C_{13}H_{10}O_5$(M^+):

246.0528; found 246.0530(48), 137(100), 136(69); ^{1}H nmr(CD_3COCD_3+D_2O): δ6.81(1H, dd, J=7.75, 7.75 Hz, H-5), 6.95(1H, d, J=8.2 Hz, H-5'), 7.09(1H, dd, J=7.75, 1.5 Hz, H-4*), 7.16(1H, dd, J=7.75, 1.5 Hz, H-6*), 7.18(1H, dd, J=8.2, 2.2 Hz, H-6'), 7.29(1H, d, J=2.2 Hz, H-2'); (*assignments interchangeable).

(S)-alcohol 126 using (S)-propylene oxide

The procedure was the same as that employed in the preparation of racemic alcohol 76. The following quantities were used. Benzamide 62: 1.355 g, 5.72×10^{-3} mol; dry tetrahydrofuran: 40 mL; TMEDA: 1.7 mL, 1.14×10^{-2} mol; s-butyllithium: 1.29 M in cyclohexane, 8.8 mL, 1.14×10^{-2} mol; freshly prepared magnesium bromide etherate: 6.5 mL, 1.72×10^{-2} mol; (S)-propylene oxide: 2.4 mL, 2.86×10^{-2} mol. After warming to room temperature overnight, the reaction mixture was diluted with saturated aqueous ammonium chloride (40 mL). The aqueous phase was extracted with ethyl acetate (3x 40 mL), dried over sodium sulphate, filtered, and evaporated in vacuo to afford crude material (2.096 g). Flash chromatography (4.5 cm column) gave recovered starting material (62, 0.234 g, 17%) from elution with Skellysolve B-ethyl acetate, 50:50, and crude (S)-alcohol 126 (1.226 g, 73%) from elution with ethyl acetate. The chiral alcohol was purified in the same way as the racemic alcohol. Thus acetylation with acetic anhydride (5 mL) and pyridine (5 mL) (reflux, 2 hours), followed by flash chromatography (4 cm

column, Skellysolve B-ethyl acetate, 50:50), gave the (S)-acetate 125 (1.291 g, representing 1.130 g (67%) of the alcohol). The (S)-acetate 125 displays tlc behaviour and a ^{1}H nmr spectrum identical with those of the racemic acetate 79. The chiral acetate was distilled (160-171°C/0.25-0.3 Torr) before optical rotation measurement. $[\alpha]_D$ -1.7° (c 5.17 $CHCl_3$).

The (S)-acetate 125 was hydrolysed in the same manner as the racemic acetate (vide supra). Thus treatment with potassium carbonate (0.55 g) in methanol (25 mL) for 27 hours afforded the (S)-alcohol 126 (0.960 g, 85%), which shows the same tlc behaviour as racemic alcohol 76. A non-polar impurity present in (S)-alcohol 126 (ca. 0.720 g) was separated by flash chromatography (3 cm column) and identified by ^{1}H nmr as a 7.5:1 mixture (5 mg) of phthalides 88 and 89. Elution with ethyl acetate afforded the repurified (S)-alcohol 126 (0.716 g). Distillation (172-184°C/0.08 Torr) afforded an analytical sample. $[\alpha]_D$ +53.0° (c 4.21 $CHCl_3$); uv(CH_3OH): 217(ϵ20,650), 222(ϵ18,585), 282(ϵ3300)nm; hreims: no M^+; m/z calcd. for $C_{14}H_{21}NO_3$ ($M^+ - C_2H_4O$): 251.1521; found 251.1518(43), 236(100), 220(41), 179(88); Analysis: calcd. for $C_{16}H_{25}NO_4$: C 65.06, H 8.53, N 4.74; found C 64.75, H 8.55, N 4.70.

(R)-alcohol 128 using (R)-propylene oxide

The procedure was the same as that employed in the preparation of racemic alcohol 76. The following quantities

were used. Benzamide 62: 1.128 g, 4.76×10^{-3} mol; dry tetrahydrofuran: 40 mL; TMEDA: 1.1 mL, 7.14×10^{-3} mol; s-butyllithium: 1.26 M in cyclohexane, 5.7 mL, 7.14×10^{-3} mol; freshly prepared magnesium bromide etherate: 5.4 mL, 1.43×10^{-2} mol; (R)-propylene oxide: 1.0 mL, 1.43×10^{-2} mol. The work-up was identical with that used for (S)-alcohol 126. Flash chromatography (4.5 cm column) gave recovered starting material (62, 0.100 g, 9%) from elution with chloroform-ethyl acetate, 80:20, and crude (R)-alcohol (0.936 g) from elution with chloroform-ethyl acetate, 50:50. As in the case of the (S)-alcohol, the (R)-alcohol was acetylated (acetic anhydride (5 mL), pyridine (5 mL), reflux, 2 hours). Flash chromatography (4.5 cm column, Skellysolve B-ethyl acetate, 50:50) of the crude acetylation products (1.105 g) afforded impure phthalide 89 (0.049 g), the (R)-acetate 127 (0.588 g, representing 0.515 g (37 %) of the (R)-alcohol), and 86 (0.048 g). The eluting solvent was changed to ethyl acetate after 86 began to elute in pure form. The (R)-acetate displays tlc behaviour and a ^{1}H nmr spectrum identical with those of the racemic acetate 79. Distillation (140-142°C/0.07 Torr) provided an analytical sample. $[\alpha]_D$ +0.8° (c 5.23 $CHCl_3$); Analysis: calcd. for $C_{18}H_{27}NO_5$: C 64.07, H 8.06, N 4.15; found C 63.86, H 7.90, N 4.11.

The (R)-acetate 127 was hydrolysed in the same manner as the racemic acetate (vide supra). Thus treatment with potassium carbonate (0.241 g) in methanol (20 mL) for 42

hours yielded the (R)-alcohol 128 (0.513 g, quantitative), which shows the same tlc behaviour as racemic alcohol 76. Distillation (156-160°C/0.075-1.0 Torr) afforded an analytical sample. $[\alpha]_D$ -47.8°C (c 4.27 $CHCl_3$); Analysis: calcd. for $C_{16}H_{25}NO_4$: C 65.06, H 8.53, N 4.74; found C 65.03, H 8.50, N 4.65.

3-methyl-3,4-dihydro-7,8-dimethoxyisocoumarin (75) via acid hydrolysis of the amide function of alcohol 76

Alcohol 76 (38 mg, 1.29×10^{-4} mol) was dissolved in 1 N hydrochloric acid (5 mL) and the magnetically stirred solution was refluxed for 2 hours. Saturated aqueous ammonium chloride (5 mL) was added and the reaction mixture was extracted with chloroform (3x 10 mL). The combined extracts were dried over sodium sulphate, filtered, and evaporated in vacuo. The crude products were subjected to flash chromatography (1 cm column) to yield a 1.5:1 mixture (5 mg) of allyl benzamide 56 (8%) and lactone 75 (7%, vide infra) by elution with chloroform-ethyl acetate, 88:12. The identitites and relative ratio of the products were determined by examination of the ^1H nmr of the mixture. Starting material 76 was isolated (30 mg, 79%) by elution with ethyl acetate.

Lactone 75 via base hydrolysis of the amide function of alcohol 76

A magnetically stirred solution of alcohol 76 (34 mg, 1.15×10^{-4} mol) in 3 N sodium hydroxide (5 mL) was refluxed for 22.5 hours. The variac setting was 75 volts (vide supra). After cooling to room temperature the reaction mixture was acidified using concentrated hydrochloric acid and saturated aqueous ammonium chloride (2 mL) was added. The solution was extracted with chloroform (3x 10 mL) and the combined extracts were dried over sodium sulphate, filtered, and evaporated to dryness in vacuo. Flash chromatography (1 cm column) of the crude products afforded lactone 75 (2.5 mg, 10%) from elution with chloroform-ethyl acetate, 92:8, and starting material 76 (27 mg, 79%) from elution with ethyl acetate.

The reaction was also carried out substituting hexamethylphosphoramide (2.5 mL, distilled at reduced pressure) for half the reaction mixture volume. The following quantities were used. Alcohol 76: 22 mg; 3 N sodium hydroxide: 2.5 mL. The reaction was refluxed (variac setting: 75 volts) for 21 hours. After cooling to room temperature the reaction mixture was acidified using concentrated hydrochloric acid and extracted with ethyl acetate (3x 10 mL). The combined ethyl acetate extracts were washed with water (2x 25 mL), dried over sodium sulphate, filtered, and evaporated to dryness in vacuo. Flash chromatography (1 cm column) afforded lactone 75 (0.5 mg, 3%, vide infra) by elution with chloroform-ethyl acetate, 92:8, and 76 (12 mg, 55%) by elution with ethyl

acetate.

Attempted hydrolysis of the amide function of 76 using a base and a high boiling solvent

Powdered potassium hydroxide (10 mg, 1.83×10^{-4} mol) was added to a solution of alcohol 76 (18 mg, 6.1×10^{-5} mol) in diethylene glycol (5 mL). The reaction mixture was refluxed for 96 hours. After cooling to room temperature the reaction mixture was acidified using concentrated hydrochloric acid. Saturated aqueous ammonium chloride (5 mL) was added and the solution was extracted with ethyl acetate (3x 10 mL). The combined ethyl acetate extracts were washed with water (2x 25), dried over sodium sulphate, filtered, and evaporated in vacuo. Tlc examination of the crude products showed that neither alcohol 76 nor lactone 75 was present.

(S)-dimethoxylactone 143

The (S)-alcohol 126 (0.124 g, 4.20×10^{-4} mol) was dissolved in 1 N hydrochloric acid (20 mL) and the magnetically stirred solution was heated at 100°C in a constant temperature oil bath for 24 hours. After cooling to room temperature the reaction mixture was diluted with saturated aqueous ammonium chloride (20 mL) and extracted with chloroform (3x 40 mL). The combined chloroform extracts were dried over sodium sulphate, filtered, and evaporated in vacuo. The crude material (0.113 g) was

subjected to flash chromatography (1 cm column, sample dissolved in chloroform; Skellysolve B-ethyl acetate-acetic acid, 75:25:1) afforded a mixture of 7-methoxylactone 143 and phthalide 88 (1 mg) and lactone 142 (46 mg, 49%, 79% based on consumed starting material). Elution with ethyl acetate yielded starting material 126 (47 mg, 38%). Compound 142 has the following physical properties. mp 83-85°C (Skellysolve B-ethyl acetate); $[\alpha]_D$ +146.3° (c 2.05 $CHCl_3$); tlc: R_f 0.19 (Skellysolve B-ethyl acetate-acetic acid, 75:25:1); ftir($CHCl_3$ cast): 1725, 1489, 1259, 1056 cm^{-1}; hreims: m/z calcd. for $C_{12}H_{14}O_4(M^+)$: 222.0892; found 222.0891(100), 189(29), 163(43), 149(23), 146(30), 135(55), 120(38), 90(52); ^1H nmr($CDCl_3$): δ1.47(3H, d, J=6.5 Hz, $CHCH_3$), 2.83(2H, d, J=6.5 Hz, CH_2), 3.89(3H, s, OCH_3), 3.95(3H, s, OCH_3), 4.53(1H, sextet, J=6.5 Hz, CH), 6.95(1H, d, J=8.5 Hz, ArH), 7.09(1H, d, J=8.5 Hz, ArH); Analysis: calcd. for $C_{12}H_{14}O_4$: C 64.85, H 6.35; found C 64.81, H 6.35.

(R)-dimethoxylactone 145

The procedure was the same as that used in the preparation of the (S)-dimethoxylactone. The following quantities were employed: (R)-alcohol 128: 0.243 g; 1 N hydrochloric acid: 40 mL. After cooling to room temperature the reaction mixture was diluted with saturated aqueous ammonium chloride (40 mL) and extracted with chloroform (3x 40 mL). The combined chloroform extracts were dried over sodium sulphate, filtered, and evaporated in vacuo to give

crude material (0.210 g). Subjection to flash chromatography (2 cm column, sample dissolved in chloroform) afforded a 5:1 mixture (4 mg) of lactone 145 and phthalide 88 by elution with Skellysolve B-ethyl acetate-acetic acid, 75:25:1, (R)-dimethoxylactone 144 (93 mg, 51%, 91% based on consumed starting material) by elution with Skellysolve B-ethyl acetate-acetic acid, 65:35:1, and recovered (R)-alcohol 128 (107 mg, 44%) by elution with ethyl acetate. Compound 144 was identical with the (S)-dimethoxylactone 142 (tlc, ftir) and has the following additional physical properties. mp 78-83°C (Skellysolve B-ethyl acetate); $[\alpha]_D$ -129.3° (c 2.05 $CHCl_3$); Analysis: calcd. for $C_{12}H_{14}O_4$: C 64.85, H 6.35; found C 64.72, H 6.50.

Demethylation of lactone 75 derived from hydrolysis of alcohol 76 under basic conditions

Dimethoxylactone 75 (2.4 mg) was dissolved in methylene chloride (1 mL) and the magnetically stirred solution was cooled to -78°C in a dry-ice bath. Excess boron tribromide (1 g/5 mL, 5 drops) was added and the reaction was allowed to warm to room temperature overnight. Water (1 mL) was added and stirring was continued for 30 minutes. The reaction mixture was diluted with saturated aqueous ammonium chloride (1 mL) and the aqueous phase was extracted with chloroform (3x 5 mL). The combined organic layers were dried over sodium sulphate, filtered, and evaporated to dryness in vacuo. Flash chromatography (0.5 cm column,

chloroform-ethyl acetate-acetic acid, 97:2:1) afforded diphenolic lactone 12 (vide supra, 1 mg)

(S)-(+)-7-hydroxymellein (133)

Dimethoxylactone 142 (40 mg) was dissolved in dry methylene chloride (8 mL) and the magnetically stirred solution was cooled to -78°C in a dry-ice bath. An excess of a solution of boron tribromide in methylene chloride (2.0 mL, 1 g/5 mL) was added and the reaction was allowed to warm to room temperature overnight. Water (10 mL) was added and stirring was continued for 30 minutes. The reaction mixture was diluted with brine (10 mL) and the aqueous phase was extracted with chloroform (3x 10 mL). The combined organic layers were dried over sodium sulphate, filtered, and evaporated to dryness in vacuo. The crude product (41 mg) was filtered through a pipette (packed with silica gel for flash chromatography) by washing with Skellysolve B (10 mL). Elution with chloroform-ethyl acetate-acetic acid, 97:2:1 (15 mL) yielded (S)-diphenolic lactone 133 (35 mg, quantitative), which was identical with the racemic lactone 12 by tlc. Compound 133 was further purified by numerous recrystallisations, and displays the following physical properties. mp: 97.5-99.5°C (Skellysolve B-ethyl acetate). $[\alpha]_D$ +92.0° (c 5.00 $CHCl_3$). Analysis: calcd. for $C_{10}H_{10}O_4$: C 61.85, H 5.19; found C 61.55, H 5.19.

(R)-(-)-7-hydroxymellein (18)

The (R)-diphenolic lactone was prepared and isolated in identical fashion to the (S) isomer. The following quantities were used: (R)-dimethoxy lactone 144: 83 mg, 3.74×10^{-4} mol; dry methylene chloride: 10 mL; boron tribromide: 1.0 M in methylene chloride, 1.0 mL, 1.12×10^{-3} mol; water: 10 mL. The (R)-diphenolic lactone 18 was obtained in quantitative yield (72 mg) and displayed identical tlc behaviour to the corresponding racemic compound 12. Synthetic (R)-(-)-7-hydroxymellein 18 was recrystallised numerous times to give an analytical sample, which has the following physical properties. mp:97.5-99.5°C (Skellysolve B-ethyl acetate)(lit.[26] mp 100-101°C); $[\alpha]_D$ -88.6° (c 5.1 $CHCl_3$)(lit. [26] optical rotation -97°+/-3° ($CHCl_3$)); Analysis: calcd. for $C_{10}H_{10}O_4$: C 61.85, H 5.19; found C 61.92, H 5.16.

Attempted preparation of lactone 75 by treatment of (S)-alcohol 126 with potassium t-butoxide

To a magnetically stirred slurry of potassium t-butoxide (48 mg, 0.122 M) in anhydrous ether (1.0 mL, used as received) was added water (2.3 µL, 0.037 M). A solution of alcohol 126 (19 mg, 0.0185 M) in anhydrous ether (2.5 mL) was added and the reaction was vigorously stirred for 24 hours at room temperature. Tlc examination of the reaction mixture revealed that only starting material was present.

The reaction was also carried out using the same procedure and substituting dry tetrahydrofuran for ether.

The following quantities were used. Potassium t-butoxide: 75 mg, 0.122 M; water: 3.7 μL, 0.037 M; alcohol 126: 30 mg, 0.0185 M. The reaction was refluxed for 48 hours. Examination of the reaction mixture by tlc showed only starting material.

The reaction was also performed using the same procedure and substituting dioxane for ether. The following quantities were used. Potassium t-butoxide: 63 mg, 0.122 M; water: 3.1 μL, 0.037 M; alcohol 126: 25 mg, 0.0185 M. After refluxing for 13.5 hours tlc analysis revealed a complex reaction mixture in which lactone 75 (vide supra) was not present.

Benzylation of (S)-alcohol 126

To a magnetically stirred slurry of sodium hydride (50 % in oil, washed with Skellysolve B; 0.078 g, 1.63×10^{-3} mol) in dry tetrahydrofuran (4 ml) was added a solution of alcohol 126 (0.096 g, 3.25×10^{-4} mol) in dry tetrahydrofuran (6 ml), under an argon atmosphere. Benzyl bromide (0.39 ml, 3.25×10^{-3} mol) was added and the reaction was stirred for 46 hours at room temperature. Brine (10 ml) was added and the aqueous phase was extracted with ethyl acetate (3x 10 ml). The combined organic layers were dried over sodium sulphate, filtered, and evaporated in vacuo. Purification of the residue by flash chromatography (2 cm column, chloroform-ethyl acetate, 85:15, changing to 50:50 as 131 finished eluting) afforded compound 131 (0.115 g, 92%).

Compound 131 exists as a pair of rotational isomers (1:1) and has the following physical properties. tlc: R_f 0.30 (chloroform-ethyl acetate, 85:15); ftir($CHCl_3$ cast): 1633, 1490, 1454, 1430, 1285, 1273, 1130, 1060 cm^{-1}; hreims: m/z calcd. for $C_{23}H_{31}NO_4(M^+)$: 385.2253; found 385.2240(0.3), 294(8), 269(9), 250(79), 236(39), 206(31), 179(40), 91(100), 72(14); ^1H nmr($CDCl_3$): δ0.99(3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 1.02(3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 1.18(3H, d, J=6 Hz, $CH\underline{CH_3}$), 1.21(3H, d, J=6 Hz, $CH\underline{CH_3}$), 1.239(3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 1.242(3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 2.50(1H, dd, J=14, 5.25 Hz), 2.55(1H, dd, J=14, 7.5 Hz), 2.79(1H, dd, J=14, 7.5 Hz), and 2.87(1H, dd, J=14, 5.5 Hz, 2x $ArCH_2$), 2.98-3.07(4H, m, 2x $\underline{CH_2}CH_3$), 3.44-3.9(6H, m, 2x $\underline{CH_2}CH_3$ and 2x $\underline{CH}(OBn)$), 3.82(6H, s, 2x OCH_3), 3.86(6H, s, 2x OCH_3), 4.36(1H, d, J=11.5 Hz), 4.47(1H, d, J=12 Hz), 4.49(1H, d, J=11.5 Hz), and 4.57(1H, d, J=12 Hz, 2x $ArCH_2O$), 6.827(1H, d, J=8.25 Hz, ArH), 6.833(1H, d, J=8.5 Hz, ArH), 6.99(1H, d, J=8.5 Hz, ArH), 7.01(1H, d, J=8.25 Hz, ArH), 7.23-7.37(10H, m, 2x C_6H_5).

Attempted amide hydrolysis of 131 using trimethyloxonium tetrafluoroborate

To a magnetically stirred slurry of trimethyloxonium tetrafluoroborate (0.017 g, $1.16x10^{-4}$ mol) in dry methylene chloride (0.5 ml) was added a solution of 131 (0.028 g, $7.27x10^{-5}$ mol) in dry methylene chloride (2 ml). The reaction was stirred for 3 hours at room temperature. Dry methylene chloride (2.5 ml) was added, followed by an ice

cold (0°C) aqueous (5 ml) solution of potassium bicarbonate (0.012 g, 1.16×10^{-4} mol). Tlc examination of the methylene chloride layer revealed that only starting material 131 was present.

A repeat reaction was allowed to stir at room temperature for 24 hours. The following quantities were used. Trimethyloxonium tetrafluoroborate: 0.0135 g, 9.14×10^{-5} mol in dry methylene chloride (2 ml); compound 131: 0.022 g, 5.71×10^{-5} mol; potassium bicarbonate: 0.009 g, 9.14×10^{-5} mol in ice cold (0°C) water (5 ml). Tlc showed only starting material to be present.

Attempted amide reduction of 76 using lithium aluminum hydride

A magnetically stirred solution of alcohol 76 (22.5 mg, 7.63×10^{-5} mol) in dry tetrahydrofuran (5 ml) was cooled to 0°C (ice bath) under an argon atmosphere. Lithium aluminum hydride (LAH, 2.1 mg, 5.49×10^{-5} mol) was added and the reaction was stirred at room temperature for 30 minutes. Hydrochloric acid (5 ml, 10% (v/v)) was added drop-wise and the aqueous phase was extracted with ether (3x 5 ml). The combined organic layers were dried over sodium sulphate, filtered, and evaporated in vacuo to give a quantitative recovery of starting material. This sample of 76 was again treated with LAH (2.1 mg) for 2 hours at room temperature. Tlc showed that no reaction had occurred, thus LAH (2.1 mg) was added and stirring was continued for 2 hours. When tlc

indicated no change in starting material, additional LAH (2.1 mg) was added and the reaction mixture was stirred for 19 hours. Examination of the reaction mixture by tlc showed only unchanged 76, thus more LAH (14.5 mg, 3.82×10^{-4} mol) was added and the reaction mixture was refluxed for 6.5 hours. However tlc indicated that no reaction had occurred.

Attempted hydrolysis of the amide function in ketone 77 using 3 N hydrochloric acid

The ketone 77 (14 mg) was dissolved in 3 N hydrochloric acid (5 mL) and the magnetically stirred solution was refluxed for 24 hours. After cooling to room temperature the reaction mixture was diluted with saturated aqueous ammonium chloride (5 mL) and extracted with ethyl acetate (3x 10 mL). The combined ethyl acetate extracts were washed with water (1x 20 mL), dried over sodium sulphate, filtered, and evaporated in vacuo to afford crude material (11 mg). Purification of the crude material by flash chromatography (0.5 cm column, chloroform-ethyl acetate, 50:50) gave starting material (6 mg, 46%) and monomethoxy ketone 102 (1 mg, 8%).

Treatment of ketone 77 (15 mg) with refluxing 6 N hydrochloric acid (3 mL) for 20.5 hours also gave 102 (7 mg, 50%) after flash chromatography. Compound 102 has the following physical properties. tlc: R_f 0.24 (chloroform-ethyl acetate, 50:50); ftir($CHCl_3$ cast): 3600-3040(b), 2965, 2930, 1708, 1625, 1491, 1275, 1060 cm^{-1};

hreims: m/z calcd. for $C_{15}H_{21}NO_4(M^+)$: 279.1471; found 279.1469(60), 222(57), 206(100), 164(71), 163(34), 136(27), 74(26), 72(66); ^1H nmr($CDCl_3$): δ1.0-1.3(6H, m, 2x $CH_2\underline{CH_3}$), 2.18(3H, s, $COCH_3$), 3.25-3.88(6H, m, $ArCH_2$ and 2x $\underline{CH_2}CH_3$), 3.92(3H, s, OCH_3), 5.74(1H, s, ArOH), 6.73(1H, d, J=8 Hz, ArH), 6.84(1H, d, J=8 Hz, ArH).

3-methyl-7,8-dihydroxyisocoumarin (13) from undistilled ketone 77

Undistilled ketone 77 (1.381 g, 4.71×10^{-3} mol) was dissolved in 6 N hydrochloric acid (100 mL) and the magnetically stirred solution was refluxed for 7 days. The colour of the solution became progessively darker (yellow to red to black). After cooling to room temperature the reaction mixture was diluted with saturated aqueous ammonium chloride (100 mL) and extracted with ethyl acetate (3x 100 mL). The combined ethyl acetate extracts were washed with water (1x 100 mL), dried over sodium sulphate, filtered, and evaporated to dryness in vacuo to give crude material (0.831 g). Purification of this material by flash chromatography (4.5 cm column, chloroform-ethyl acetate-acetic acid, 98.5:1:0.5) afforded a 2.5:1 mixture (0.205 g) of isocoumarin 13 and 12.

Isocoumarin 13 from distilled ketone 77

Distilled ketone 77 (0.244 g, 8.33×10^{-4} mol) was dissolved in 6 N hydrochloric acid (20 mL) and the

magnetically stirred solution was refluxed for 6 days. The reaction gradually became coloured. Upon cooling to room temperature saturated aqueous ammonium chloride (20 mL) was added and the products were isolated by extraction with ethyl acetate (3x 20 mL). The combined ethyl acetate extracts were washed with water (1x 20 mL), dried over sodium sulphate, filtered, and evaporated to dryness *in vacuo* to give crude material (0.161 g). Purification of this material by flash chromatography (1 cm column, chloroform-ethyl acetate-acetic acid, 98.5:1:0.5) afforded isocoumarin 13 (0.039 g) contaminated with a small amount (*ca*. 5%) of the corresponding 7-methoxyisocoumarin. The mixture was dissolved in methylene chloride (5 mL) and the solution was cooled to -78°C in a dry-ice bath. Excess boron tribromide (3 mL, 1 g/5 mL) was added and the reaction was allowed to warm to room temperarure overnight. Water (3 mL) was added and stirring was continued for 30 minutes. Saturated aqueous ammonium chloride (5 mL) was added and the aqueous phase was extracted with chloroform (3x 10 mL). The combined organic layers were dried over sodium sulphate, filtered and evaporated to dryness *in vacuo* to yield isocoumarin 13 (0.039 g, 24% from ketone 77). Compound 13 has the following physical properties. mp: 153-154°C (Skellysolve B-ethyl acetate); tlc: R_f 0.62 (chloroform-methanol, 90:10); ftir($CHCl_3$ cast): 3410, 2960(w), 2922(w), 1682, 1637, 1269, 1124, 841 cm^{-1}; uv(CH_3OH): 242(ϵ6100), 272(ϵ5500), 372(ϵ4200)nm; hreims: m/z

calcd. for $C_{10}H_8O_4(M^+)$: 192.0423; found 192.0417(100), 163(18), 150(12), 121(24); ^1H nmr($CDCl_3$): δ2.27(3H, d, J=1 Hz, CH_3), 5.68(1H, bs, ArOH), 6.26(1H, d, J=1 Hz, CH), 6.81(1H, d, J=8 Hz, ArH), 7.31(1H, d, J=8 Hz), 10.71(1H, bs, peri ArOH); ^{13}C nmr(CD_3OD): δ18.9(CH_3); 105.5, 116.6, 125.3(CH); 107.1, 130.9, 145.1, 149.7, 152.6(C); 168.5(C=O); Analysis: calcd. for $C_{10}H_8O_4$: C 62.50, H 4.20; found C 62.50, H 4.27.

Hydrolysis of the amide function of ketone 77 using hydrochloric acid containing BHT

To a solution of ketone 77 (0.234 g, 7.99×10^{-4} mol) in 6 N hydrochloric acid (20 mL) was added butylated hydroxytoluene (106, BHT, 0.176 g, 7.99×10^{-4} mol). The reaction mixture was refluxed for 7 days, during which time its colour gradually became orange, and finally dark red. After cooling to room temperature the reaction mixture was diluted with saturated aqueous ammonium chloride (20 mL) and extracted with chloroform (3x 40 mL). The combined chloroform extracts were dried over sodium sulphate, filtered, and evaporated to dryness in vacuo to give crude material (93 mg), purple in colour. The crude was subjected to treatment with boron tribromide. Thus boron tribromide in methylene chloride (1 g/5 mL, 3 mL) was added to a solution of the crude in methylene chloride (10 mL). The reaction was magnetically stirred for 6.5 hours at room temperature. Water (10 mL) was then added, and stirring was

continued for 30 minutes. Brine (10 mL) was added and the aqueous phase was extracted with chloroform (3x 20 mL). The combined chloroform extracts were dried over sodium sulphate, filtered, and evaporated to dryness *in vacuo* to give crude material (90 mg). Purification of the crude material by flash chromatography (2 cm column, chloroform-ethyl acetate-acetic acid, 98.5:1:0.5) gave a 7.5:1 mixture (6 mg, 4%) of 13 and 12.

N,N-diethyl-2,3-hydroxy-6-(2-propanoyl)benzamide (103)

To a magnetically stirred solution of ketone 77 (0.116 g, 3.96×10^{-4} mol) in methylene chloride (5 mL) was added boron tribromide (1 g/5 mL in methylene chloride, 1.0 mL, 7.92×10^{-4} mol). The reaction was stirred overnight at room temperature. Water (3 mL) was added and stirring was continued for 30 minutes. Saturated aqueous ammonium chloride (2 mL) was added and the aqueous phase was extracted with chloroform (3x 10 mL). The combined organic layers were dried over sodium sulphate, filtered, and evaporated *in vacuo* to afford crude material (0.121 g) as a yellow foam. The crude material was subjection to flash chromatography (2 cm column, chloroform-methanol, 92.5:7.5) to give 103 (0.062 g, 59%). Compound 103 has the following physical properties. tlc: R_f 0.36 (chloroform-methanol, 90:10); ir($CHCl_3$): 3560-3200(b), 2935, 1710, 1580 cm^{-1}; hreims: m/z calcd. for $C_{14}H_{19}NO_4$ (M^+): 265.1314; found 265.1312(29), 208(17), 192(55), 150(64), 149(31), 74(100),

72(41), 58(37); ^{1}H nmr($CDCl_3$): δ1.16(6H, bs, 2x $CH_2\underline{CH_3}$), 2.22(3H, s, $COCH_3$), 3.38(4H, bs, 2x $\underline{CH_2}CH_3$), 3.51(2H, s, $ArCH_2$), 6.55(1H, d, J=8 Hz, ArH), 6.73(1H, d, J=8 Hz, ArH), 7.87(1H, bs, ArOH), 8.03(1H, bs, ArOH).

Attempted cyclisation of 103 to 13 using a high boiling solvent

A magnetically stirred solution of crude diphenolic ketone 103 (20.5 mg) in xylenes (5 mL, bp 136-145°C) was refluxed for 15 hours. Methanol was added and the solvents were removed in vacuo. Tlc analysis indicated the possible presence of 13, thus the crude was subjected to flash chromatography (1 cm column, chloroform-ethyl acetate-acetic acid, 97:2:1). However comparative tlc showed that none of the isolated products was compound 13.

N,N-diethyl-2,3-dihydroxybenzamide (109)

A magnetically stirred solution of benzamide 62 (58 mg, 2.45×10^{-4} mol) in dry methylene chloride (10 mL) was cooled to -78°C in a dry-ice bath, under an argon atmosphere. Boron tribromide in methylene chloride (1 g/5 mL, 1.0 mL) was added and the reaction mixture was allowed to warm to room temperature overnight. Water (5 mL) was added and stirring was continued for 30 minutes. The reaction was diluted with brine and the aqueous phase extracted with chloroform (3x 5 mL). The combined organic layers were dried over sodium sulphate, filtered, and evaporated to

dryness in vacuo to yield dihydroxy benzamide 109 (49 mg, 96%). Compound 109 has the following physical properties. mp 137-139°C (benzene-ethyl acetate); tlc: R_f 0.28 (chloroform-methanol, 95:5); ftir(CH_2Cl_2): 3550-2400(b), 1626(m), 1582(s), 1496, 1467, 1442, 1332, 1212 cm^{-1}; uv(CH_3OH): 217(ϵ5800), 284(ϵ1600)nm; hreims: m/z calcd. for $C_{11}H_{15}NO_3(M^+)$: 209.1029; found 209.1050(55), 137(45), 136(49), 74(39), 72(44), 58(100); ^1H nmr($CDCl_3$): δ1.29(6H, t, J=7.5 Hz, 2x CH_3), 3.55(4H, q, J=7.5 Hz, 2x CH_2), 5.73(1H, s, ArOH), 6.75(1H, dd, J=7.5, 7.5 Hz, ArH), 6.85(1H, dd, J=7.5 1.5 Hz, ArH), 6.98(1H, dd, J=7.5, 1.5 Hz, ArH), 10.23(1H, s, peri ArOH).

Isocoumarins 13 and 108 from pyrolysis of crude 103

Impure keto-diphenol 103 (tlc analysis showed presence of a less polar component (R_f 0.56, chloroform-methanol, 90:10), presumably the 7-methoxy-keto phenol 24, vide supra) was prepared (vide supra) from ketone 77 (63 mg, 2.15×10^{-4} mol). Impure 103 was heated to 189°C for 20 minutes in a bulb-to-bulb distillation apparatus, resulting in the appearance of crystalline material in the bulb of "distilled" material. The temperature was increased to 250°C and maintained for 30 minutes. Subjection of the "distilled" material (35 mg) to flash chromatography (1 cm column, chloroform-ethyl acetate-acetic acid, 98.5:1:0.5) yielded 13 (5 mg, 12% from 77) and 108 (3 mg, 7% from 77). Compound 108 has the following physical properties. tlc: R_f

0.51 (chloroform-ethyl acetate-acetic acid, 97:2:1); ^{1}H nmr($CDCl_3$): δ2.26(3H, s, vinylic CH_3), 3.95(3H, s, OCH_3), 6.22(1H, s, CH), 6.79(1H, d, J=8 Hz, ArH), 7.25(1H, d, J=8 Hz, ArH), 11.20(1H, s, ArOH).

Isocoumarin 13 from ketone 77 (pyrolysis route)

Distilled ketone 77 (1.705 g, 5.82×10^{-3} mol) was dissolved in dry methylene chloride (25 mL) under an argon atmosphere. The magnetically stirred solution was cooled to -78°C in a dry-ice bath and boron tribromide (1.0 M in methylene chloride, 5.8 mL, 5.82×10^{-3} mol) was added. The reaction was allowed to warm to room temperature overnight. Water (15 mL) was added and stirring at room temperature was continued for 30 minutes. The reaction mixture was diluted with brine (30 mL) and the aqueous phase was extracted with chloroform (3x 30 mL). The combined organic extracts were dried over sodium sulphate, filtered, and evaporated to dryness in vacuo to afford a mixture of impure keto-diphenol 103 and the corresponding 7-methoxy-keto phenol 24 (vide supra) as a yellow foam (1.669 g). The foam was crushed to a powder and a portion (1.614 g) was heated at 230°C (0.25-0.3 Torr) for 30 minutes in a bulb-to-bulb distilllation apparatus. The "distilled" material (0.470 g) was subjected to flash chromatography (3 cm column, chloroform-ethyl acetate-acetic acid, 98.5:1:0.5) to give a mixture of 13 and 108 (0.362 g). The mixture was dissolved in methylene chloride (40 mL). Boron tribromide (1.0 M in

methylene chloride, 3.5 mL) was added and the reaction was magnetically stirred overnight at room temperature. Water (40 mL) was added and stirring was continued for 30 minutes. The reaction mixture was diluted with brine (40 mL) and the aqueous phase was extracted with chloroform (3x 80 mL). The combined organic layers were dried over sodium sulphate, filtered, and evaporated to dryness *in vacuo* to afford isocoumarin 13 (0.351 g, 31% from 77).

N,N-diethyl-2,3-dihydroxy6-(2-bromopropyl)benzamide (135)

(S)-alcohol 126 (0.375 g, 1.27×10^{-3} mol) was dissolved in dry methylene chloride (40 mL) under an argon atmosphere. The magnetically stirred solution was cooled to -78°C in a dry-ice bath. Boron tribromide (1.0 M in methylene chloride, 3.4 mL, 3.43×10^{-3} mol) was added and the reaction was allowed to warm to room temperature overnight. Water (10 mL) was added and stirring was continued for 30 minutes. The reaction mixture was diluted with brine (30 mL) and the aqueous phase was exytracted with chloroform (3x 30 mL). The combined organic layers were dried over sodium sulphate, filtered, and evaporated to dryness *in vacuo* to give impure (tlc) (S)-bromide 135 (0.459 g) as a white foam. The foam was stored *in vacuo* in the dark. Impure 135 has the following physical properties. $[\alpha]_D$ +54.6° (c 2.40 $CHCl_3$); tlc: R_f 0.37 (chloroform-ethyl acetate, 50:50); ftir($CHCl_3$ cast): 3550-2300(b), 1612, 11587, 1499, 1289 cm^{-1}; hreims: m/e calcd. for $C_{14}H_{20}NO_3{}^{81}Br$(M+2): 331.0606; found

331.0605(23); calcd. for $C_{14}H_{20}NO_3{}^{79}Br(M^+)$: 329.0626; found 329.0624(23), 258(21), 256(22), 250(38), 249(23), 177(36), 149(29), 74(100), 72(87), 58(45); cims: 332(M+2+1), 330(M+1), 250(M+2 $-H^{81}Br$ and $M^+ - H^{79}Br$); ^1H nmr($CDCl_3$): δ1.0-1.5(6H, bs, 2x $CH_2\underline{CH_3}$), 1.68(3H, d, J=6.5 Hz, $CHBr\underline{CH_3}$), 2.7-4.05(6H, m, 2x $\underline{CH_2}CH_3$, $ArCH_2$), 4.33(1H, sextet, J=6.5 Hz, CHBr), 6.55(1H, d, J=8 Hz, ArH), 6.69(1H, d, J=8 Hz, ArH), 7.61(2H, bs, 2x ArOH).

Treatment of acetate 79 with boron tribromide

Acetate 79 (26 mg) was dissolved in dry methylene chloride (3 mL) under an argon atmosphere. The magnetically stirred solution was cooled to -78°C in a dry-ice bath. Boron tribromide (1 g/5 mL, 1.0 mL) was added and the reaction was allowed to warm to room temperature overnight. Water (2 mL) was added and stirring was continued for 30 minutes. The reaction mixture was diluted with brine (5 mL) and the aqueous phase was extracted with chloroform (3x 5 mL). The combined organic extracts were dried over sodium sulphate, filtered, and evaporated to dryness in vacuo. The crude product (27 mg) displays identical tlc behaviour to 135 and gives the same major peaks (m/z 332, 330, 250) as 135 by cims. The ^1H nmr of the crude also possesses the same major signals as that of 135.

Acetylation of bromide 135

Crude diphenolic bromide 135 (21 mg) was dissolved in acetic anhydride (2 mL) and pyridine (2 mL). The solution was magnetically stirred overnight at room temperature in the dark. Toluene was added and the solvents were removed in vacuo. The crude material (25 mg) was subjected to flash chromatography (1 cm column, Skellysolve B-ethyl acetate, 50:50) to yield diacetyl bromide 134 (21 mg). Compound 134 exists as a pair of rotational isomers (1.4:1) and has the following physical properties. tlc: R_f 0.36 and 0.31 (Skellysolve B-ethyl acetate, 50:50); ftir($CHCl_3$ cast): 1781, 1634, 1205, 1178 cm^{-1}; hreims: m/z calcd. for $C_{18}H_{24}NO_5{}^{81}Br$(M+2): 415.0817; found 415.0836(0.4); calcd. for $C_{18}H_{24}NO_5{}^{79}Br$($M^+$): 413.0837; found 413.0849(0.4), 373(41), 371(41), 334(5), 331(47), 329(48), 292(13), 258(19), 256(19), 250(37), 177(35), 176(32), 74(100), 72(60), 58(27); cims: m/z 433(M+2+18), 431(M+18); ^1H nmr($CDCl_3$): δ1.06(3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 1.07(3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 1.24(3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 1.25(3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 1.64(3H, d, J=6.5 Hz, $CHBr\underline{CH_3}$), 1.72(3H, d, J=6.5 Hz, $CHBr\underline{CH_3}$), 2.232(3H, s, $COCH_3$), 2.234(3H, s, $COCH_3$), 2.26(6H, s, 2x $COCH_3$), 2.96-3.22(8H, m, 2x $ArCH_2$, 2x $\underline{CH_2}CH_3$), 3.46-3.70(4H, m, 2x $\underline{CH_2}CH_3$), 4.37(2H, m, 2x CHBr), 7.15(1H, d, J=8 Hz, ArH), 7.20(1H, d, J=8 Hz, ArH), 7.22(1H, d, J=8 Hz, ArH), 7.25(1H, d, J=8 Hz, ArH).

Partially racemic (S)-lactone 133 from diphenolic bromide 135

Crude diphenolic bromide 135 (42 mg) was dissolved in chloroform (3 mL). The magnetically stirred solution was irradiated with an ordinary 100 watt light bulb (ca. 6 inches from the flask) for 7 days at room temperature. The solvent was removed in vacuo. The residue was subjected to flash chromatography (1 cm column) to afford (S)-lactone 133 (9 mg, 39% from (S)-alcohol 126, $[\alpha]_D$ +45.8° (c 0.72 $CHCl_3$)) by elution with chloroform-ethyl acetate, 97:3 and starting material (135, 7 mg) by elution with chloroform-ethyl acetate, 60:40.

The same reaction was observed to proceed in the dark. Thus the crude diphenolic bromide (16 mg), when stirred in chloroform (1 mL) in the dark for 7 days ~~at~~ room temperature, gave the lactone (3 mg, 33% from the alcohol) after purification by flash chromatography.

At higher temperature the reaction occurred more quickly. Thus a magnetically stirred solution of crude bromide 135 (23 mg) in chloroform (12.5 mL) was refluxed for 48 hours. Tlc analysis revealed that the bromide was almost completely consumed. Evaporation of the solvent in vacuo and purification of the residue by flash chromatography yielded (S)-lactone 133 (6 mg, 50% from (S)-alcohol 126).

Treatment of diphenolic bromide 135 with silver nitrate

Crude bromide 135 (30.5 mg) was dissolved in 95% ethanol (1.5 mL), giving a bright yellow solution. Upon addition of finely ground silver nitrate (16 mg) to the

magnetically stirred solution, a precipitate (silver bromide) formed and the solution became almost colourless. The reaction was stirred in the dark at room temperature for 7 days. Filtration afforded silver bromide (13 mg, 81% from alcohol 126). Evaporation of the solvent in vacuo gave crude reaction products (29 mg). Subjection to flash chromatography (1 cm column, sample dissolved in chloroform) yielded lactone 133 (5 mg, 31% from alcohol 126) from elution with Skellysolve B-ethyl acetate-acetic acid, 75:25:1 and impure ethyl ether 136 (10 mg) from elution with Skellysolve B-ethyl acetate-acetic acid, 25:75:1. Impure compound 136 exists as rotational isomers and has the following physical properties. tlc: R_f 0.26 (Skellysolve B-ethyl acetate-acetic acid, 65:35:1, 3x); ftir($CHCl_3$ cast): 3600-2400(b), 1624, 1604, 1584, 1499, 1292 cm^{-1}; hreims: m/z calcd. for $C_{16}H_{25}NO_4(M^+)$: 295.1784; found 295.1787(42), 250(5), 223(46), 208(73), 206(21), 150(56), 74(100), 73(45), 72(60), 58(29).

Acetylation of diphenolic ethyl ether 136

Impure diphenolic ethyl ether 136 (5 mg) was dissolved in acetic anhydride (0.5 mL) and pyridine (0.5 mL). The solution was magnetically stirred at room temperature for 17.5 hours. Toluene was added and the solvents were removed in vacuo. The crude products were passed through a pipette containing flash silica gel, first washing with Skellysolve B (5 mL), then eluting with ethyl acetate (10 mL) to give

impure diacetyl ethyl ether 137 (6 mg). Impure 137 exists as rotational isomers and has the following physical properties. tlc: R_f 0.25 and 0.20 (Skellysolve B-ethyl acetate, 50:50); ftir($CHCl_3$ cast): 1780, 1636, 1205, 1179 cm^{-1}; hreims: m/z calcd. for $C_{20}H_{29}NO_6(M^+)$: 379.1995; found 379.1976(0.5), 350(5), 337(8), 307(83), 292(11), 265(20), 250(100), 248(42), 223(42), 74(39), 73(34), 72(46), 58(20).

Treatment of diphenolic bromide 135 with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)

Crude diphenolic bromide 135 (30 mg) was dissolved in dry benzene (10 mL) under an argon atmosphere. DBU (38 μL was added and the magnetically stirred solution was refluxed for 19 hours. Upon cooling to room temperature a fine white precipitate was evident. The reaction mixture was washed with 10% (v/v) hydrochloric acid (1x 10 mL) causing the precipitate to dissolve. The benzene layer was washed with brine (1x 10 mL), dried over sodium sulphate, filtered, and evaporated in vacuo to give a 2:1 mixture (7 mg) of lactone 133 and olefin 138.

Benzylation of isocoumarin 13

Isocoumarin 13 (50 mg, 2.60×10^{-4} mol) was dissolved in dry acetone (15 mL). Anhydrous potassium carbonate (36 mg, 2.60×10^{-4} mol) was added, followed by benzyl bromide (31 μL, 2.60×10^{-4} mol, used as received). The reaction was magnetically stirred for 48 hours at room temperature, then

heated at 56°C in a constant temperature oil bath for 48 hours. The reaction mixture was evaporated to dryness *in vacuo*, then redissolved in chloroform. Water (20 mL) was added and the aqueous phase was acidified using 5% (v/v) hydrochloric acid. The aqueous phase was extracted with chloroform (3x 20 mL). The combined chloroform layers were dried over sodium sulphate, filtered, and evaporated to dryness *in vacuo*. The crude (110 mg) was subjected to flash chromatography (2 cm column, sample dissolved in methylene chloride) to yield unidentified material (34 mg), 7-benzyloxyisocoumarin 110 (23 mg, 32%) by elution with toluene-acetone, 99.5:0.5, and 7,8-dibenzyloxyisocoumarin 112 (20.5 mg, 21%) and 8-benzyloxyisocoumarin 111 (6 mg, 8%) by elution with toluene-acetone, 99:1. Compound 110 has the following physical properties. mp 119-120°C (Skellysolve B-ethyl acetate); tlc: R_f 0.59 (benzene-acetone, 95:5); ftir($CHCl_3$ cast): 3600-2700(b), 1691, 1657, 1452, 1315, 1255, 1155, 1116, 750 cm^{-1}; hreims: m/z calcd. for $C_{17}H_{14}O_4(M^+)$: 282.0892; found 282.0892(27), 191(27), 91(100); ^1H nmr($CDCl_3$): δ2.25(3H, s, CH_3), 5.22(2H, s, $ArCH_2$), 6.19(1H, s, CH), 6.70(1H, d, J=8.5 Hz, ArH), 7.2-7.5(6H, m, 6x ArH), 11.24(1H, s, ArOH).

Compound 112 has the following physical properties. mp 122-123°C (ethyl acetate); tlc: R_f 0.49 (benzene-acetone, 95:5); ftir($CHCl_3$ cast): 3029, 2917, 1734, 1676, 1497, 1279, 1160, 1029, 983, 747, 697 cm^{-1}; hreims: m/z calcd. for $C_{24}H_{20}O_4(M^+)$: 372.1362; found 372.1357(3), 281(8), 192(4),

91(100); ^{1}H nmr($CDCl_3$): δ2.15(3H, s, CH_3), 5.07(2H, s, $ArCH_2$), 5.11(2H, s, $ArCH_2$), 6.05(1H, s, CH), 6.93(1H, d, J=8.5 Hz, ArH), 7.25-7.42(9H, m, 9x ArH), 7.57(1H, d, J=7.5 Hz, ArH), 7.58(1H, d, J=8 Hz, ArH).

Compound 111 has the following physical properties. mp 141-143°C (Skellysolve B-ethyl acetate); tlc: R_f 0.36 (benzene-acetone, 95:5); ftir($CHCl_3$ cast): 3550-2700(b), 1727, 1704, 1685, 1492, 1304, 1283, 1155 cm^{-1}; hreims: m/z calcd. for $C_{17}H_{14}O_4$(M^+): 282.0892; found 282.0890(28), 191(28), 163(18), 91(100); ^{1}H nmr($CDCl_3$): δ2.26(3H, s, CH_3), 5.14(2H, s, $ArCH_2$), 5.87(1H, bs, ArOH), 6.18(1H, s, CH), 7.02(1H, d, J=8.5 Hz, ArH), 7.24-7.66(6H, m, 6x ArH).

Attempted lactol formation from compound 112

Aqueous sodium hydroxide (0.02 N, 6 mL) was added to dibenzyl isocoumarin 112 (19.5 mg). The solution was boiled for 30 minutes, however most of 112 failed to dissolve. Upon cooling to room temperature chloroform (10 mL) was added and the reaction mixture was cooled to 0°C in an ice bath. The pH was adjusted to ca. 4 using 5% (v/v) hydrochloric acid. The aqueous phase was extracted with chloroform (3x 10 mL). The combined chloroform extracts were dried over sodium sulphate, filtered, and evaporated to dryness in vacuo at room temperature. Examination of the crude material (18.5 mg) by tlc revealed mostly starting material 112.

The crude material was resubjected to boiling, aqueous sodium hydroxide (0.02 N, 6 mL) containing 0.5 mL dimethoxyethane. The milky reaction became clearer upon refluxing. Crude material was isolated as indicated in the above procedure, however tlc analysis showed 112 to be the only compound present in significant quantity.

The crude material was dissolved in aqueous sodium hydroxide (0.02 N, 5 mL) and methanol (5 mL). The solution was refluxed for 20 minutes, then allowed to cool to room temperature. Upon cooling to 0°C in an ice bath, the pH of the solution was lowered to 5.5-6. Extraction with chloroform (vide supra) afforded crude material (17.5 mg). Purification of the crude by flash chromatography (1 cm column) gave starting material 112 by elution with toluene-acetone, 98:2, and keto-ester 113 (3 mg, 14%) by elution with toluene-acetone, 96:4. Compound 113 has the following physical properties. tlc: R_f 0.21 (benzene-acetone, 96:4); ftir($CHCl_3$ cast): 2923, 1726, 1712, 1485, 1263, 1051 cm^{-1}; hreims: m/z calcd. for $C_{25}H_{24}O_5(M^+)$: 404.1624; found 404.1635(4), 313(3), 282(3), 91(100); ^1H nmr($CDCl_3$): δ2.13(3H, s, $COCH_3$), 3.66(2H, s, $ArCH_2CO$), 3.77(3H, s, OCH_3), 5.11(2H, s, $ArCH_2O$), 5.14(2H, s, $ArCH_2O$), 6.89(1H, d, J=8.5 Hz, ArH), 7.02(1H, d, J=8.5 Hz, ArH), 7.21-7.49(10H, m, 10x ArH).

Lactol formation from 7-benzyloxyisocoumarin 110

Monobenzyloxyisocoumarin 110 (23 mg, 8.16×10^{-5} mol) was dissolved in aqueous sodium hydroxide (0.02 N, 8 mL). The solution was boiled for 20 minutes, then allowed to cool to room temperature. Ether (10 mL) was added and the reaction mixture was cooled to 0°C. Following adicification to ca. pH 5.5 (5% (v/v) hydrochloric acid) the aqueous phase was extracted with ether (3x 10 mL). The pH of the aqueous layer was lowered to ca. 2.5 and re-extraction with ether (3x 10 mL) caused the previously milky aqueous layer to become clear. Each set of combined ether extracts was dried over sodium sulphate (1 hour) and filtered. The solvent was removed in vacuo at a temperature of <30°C. The first ether extract gave an insignificant quantity of crude material, however the second extract afforded lactol 114 (21.5 mg, 88%). Compound 114 has the following physical properties. mp: 132-133°C (benzene-ethyl acetate); tlc: R_f 0.34 (chloroform-methanol-acetic acid, 94:5:1); ftir($CHCl_3$ cast): 3600-2800(b), 1650, 1446, 1361, 1244, 1073, 1042, 1022, 793 cm^{-1}; hreims: m/z calcd. for $C_{17}H_{16}O_5(M^+)$: 300.0998; found 300.0998(8), 282(4), 209(1), 191(5), 91(100); ^1H nmr($CDCl_3$): δ1.78(3H, s, CH_3), 3.17(2H, s, $\underline{CH_2}C(OH)CH_3$), 5.17(2H, s, $ArCH_2O$), 6.61(1H, d, J=8 Hz, ArH), 7.03(1H, d, J=8 Hz, ArH), 7.29-7.50(5H, m, 5x ArH), 11.20(1H, bs, ArOH).

3-methyl-3,4-dihydro-3,7,8-trihydroxyisocoumarin (15)

Monobenzyloxylactol 114 (16.5 mg, 5.50×10^{-5} mol) was dissolved in ethyl acetate (5 mL). Palladium (5% w/w) on

charcoal (16.5 mg) was added and the solution was magnetically stirred for 7 hours under a positive pressure (18 psi) of hydrogen. Removal of the palladium catalyst by filtration through Celite and evaporation of the solvent *in vacuo* (<30°C) afforded the lactol **15** (11 mg, 95%). Compound **15** has the following physical properties. mp 148-149.5°C (benzene-ethyl acetate); tlc: R_f 0.30 (Skellysolve B-ethyl acetate-acetic acid, 25:75:1); ftir(CH_3COCH_3 cast): 3600-2400(b), 1669, 1453, 1272, 1236, 1173, 1072; uv(CH_3OH): 230(ϵ7000), 256(ϵ6000), 334(ϵ3500)nm; hreims: m/z calcd. for $C_{10}H_{10}O_5(M^+)$: 210.0528; found 210.0534(53), 192(80), 150(100), 149(78), 122(26), 121(28); ^1H nmr($CDCl_3$-acetone-d_6, 2:1): δ1.79(3H, s, CH_3), 3.27(2H, bs, CH_2), 6.63(1H, d, J=8 Hz, ArH), 7.04(1H, d, J=8 Hz, ArH); ^{13}C nmr($CDCl_3$-CD_3SOCD_3): δ28.3(CH_3); 42.2(CH_2); 120.2, 120.6(CH); 110.2, 127.8(2x), 144.7, 150.8(C); 171.4(C=O); Analysis: calcd. for $C_{10}H_{10}O_5$: C 57.14, H 4.80; found C 57.35, H 4.85.

Lactol **14** directly from isocoumarin **13**

Isocoumarin **13** (0.101 g, 5.26×10^{-4} mol) was dissolved in aqueous sodium hydroxide (0.02 N, 33 mL). The solution was refluxed for 20 minutes, then allowed to cool to room temperature. Ethyl acetate (35 mL) was added and the reaction mixture was cooled to 0°C in an ice bath. Hydrochloric acid (5% v/v) was added to adjust the pH to *ca.* 5.5. The aqueous phase was extracted with ethyl acetate (2x

35 mL). The pH was lowered to ca. 2.5 using hydrochloric acid (5% v/v). Saturated aqueous ammonium chloride (35 mL) was added and the aqueous layer was reextracted with ethyl acetate (3x 35 mL). Each of the two sets of combined ethyl acetate extracts was dried over sodium sulphate (1 hour), filtered, and evaporated to dryness in vacuo at a temperature of <30°C. Dry flash chromatography (1 cm column, Skellysolve B-ethyl acetate-acetic acid, 50:50:1) of the crude material (25 mg) from extraction at ca. pH 5.5 afforded 13 (1 mg) and lactol 15 (17 mg). The crude material from the extraction at ca. pH 2.5 was filtered through a pipette containing flash silica gel (eluting with Skellysolve B-ethyl acetate-acetic acid, 25:75:1). Toluene was added and the solvents were removed (<30°C) in vacuo to yield lactol 15 (84 mg). The total yield of lactol 15 was 101 mg, 92%.

Attempted allylic bromination of isocoumarin 13

N-Bromosuccinimide (62 mg, 3.48×10^{-4} mol, freshly recrystallised from nitromethane, pulverised) was added to dry carbon tetrachloride (10 mL) and a mixture (7:3) of 13 and 12 (48 mg). The magnetically stirred solution was irradiated using a 100-watt light source and refluxed for 1.5 hours, resulting in a bright orange reaction mixture. The solid precipitate (succinimide) was removed by filtration and washed with more hot carbon tetrachloride. The filtrate was evaporated to dryness in vacuo to afford

crude material which was subjected to flash chromatography (2 cm column). Elution with chloroform-ethyl acetate-acetic acid, 98.5:1:0.5 gave a mixture (18 mg) containing bromide 118 and traces of 12 and 13. Elution with chloroform-ethyl acetate-acetic acid, 97:2:1 afforded an impure dibromide. Compound 118 has the following physical properties. tlc: R_f 0.65 (chloroform-methanol, 90:10); hreims: m/z calcd. for $C_{10}H_7O_4{}^{81}Br$(M+2): 271.9508; found 271.9511(99); calcd. for $C_{10}H_7O_4{}^{79}Br(M^+)$: 269.9527; found 269.9526(100), 191(24); ^1H nmr($CDCl_3$): δ2.30(3H, s, CH_3), 5.78(1H, bs, ArOH), 6.52(1H, CH), 7.50(1H, s, ArH), 11.14(1H, s, peri ArOH).

The dibromide has the following physical properties. tlc: R_f 0.47 (chloroform-methanol, 90:10); hreims: m/z calcd. for $C_{10}H_6O_4{}^{81}Br_2$(M+4): 351.8592; found 351.8600(11); calcd. for $C_{10}H_6O_4{}^{81}Br{}^{79}Br$(M+2): 349.8612; found 349.8623(21); calcd. for $C_{10}H_6O_4{}^{79}Br(M^+)$: 347.8632; found 347.8639(10).

Acetylation of isocoumarin 13

Isocoumarin 13 (39 mg, 2.03×10^{-4} mol) was dissolved in acetic anhydride (2 mL) and pyridine (2 mL), and the solution was magnetically stirred at room temperature for 13 hours. Toluene was added and the solvents were removed in vacuo to give crude material (60 mg). Flash chromatography (1 cm column, chloroform-ethyl acetate-acetic acid, 97:2:1) of the crude yielded diacetylisocoumarin 119 (52 mg, 93%). Compound 119 has the following physical properties. mp:

168-169°C (ethyl acetate); tlc: R_f 0.23 (chloroform-ethyl acetate, 97:3); ftir($CHCl_3$ cast): 1777, 1736, 1726, 1496, 1204, 1186, 1174, 1151, 1021 cm^{-1}; hreims: m/z calcd. for $C_{14}H_{12}O_6(M^+)$: 276.0634; found 276.0634(4), 234(4), 192(100); ^1H nmr($CDCl_3$): δ2.21(3H, s, vinylic CH_3), 2.32(3H, s, $OCOCH_3$), 2.41(3H, s, $OCOCH_3$), 6.20(1H, s, CH), 7.18(1H, d, J=8.5 Hz, ArH), 7.49(1H, d, J=8.5 Hz, ArH).

Attempted allylic oxidation of diacetylisocoumarin 119

Selenium dioxide (5 mg, 4.71×10^{-5} mol) was added to a solution of diacetylisocoumarin 119 (10 mg, 3.62×10^{-5} mol) in acetic acid (5 mL). The magnetically stirred solution was refluxed for 3 hours. Upon cooling to room temperature the reaction mixture was diluted with water (5 mL) and extracted with ether (3x 10 mL). The combined ether extracts wrere washed with aqueous sodium bicarbonate (5% w/v, 1x 10 mL) and brine (1x 10 mL), dried over sodium sulphate, filtered, and evaporated in vacuo. Examination of the crude material by tlc showed 119 to be present, thus the crude was resubjected to the same reaction conditions, using 6 mg (5.43×10^{-5} mol) selenium dioxide. The solution was refluxed for 22.5 hours. The crude products were isolated in the same way as before. Flash chromatography (1 cm column, chloroform-ethyl acetate, 95:5) of the crude gave impure 119 (3 mg) and a mixture of 119 and an unidentified product (3 mg). The ^1H nmr spectrum of the latter mixture indicated that the unidentified product does not possess the

structure of the desired product.

Allylic bromination of diacetylisocoumarin 119

N-Bromosuccinimide (18 mg, 9.97×10^{-5} mol, recrystallised from nitromethane, pulverised) was added to 119 (25 mg, 9.06×10^{-5} mol) and dry carbon tetrachloride (12 mL). The magnetically stirred solution was refluxed for 6 hours while irradiating the reaction mixture with a 100-watt light source. After cooling to room temperature the reaction mixture was filtered and evaporated to dryness *in vacuo*. ^{1}H nmr analysis of the crude products indicated a mixture (*ca.* 1:1) of starting material and the desired monobromide. The crude was resubjected to the above reaction conditions, using N-bromosuccinimide (18 mg) and dry carbon tetrachloride (12 mL). After 2 hours the reaction mixture was allowed to cool to room temperature and was filtered, washing the solid residue (succinimide) with more hot carbon tetrachloride. The filtrate was evaporated to dryness *in vacuo*. The crude material obtained was refiltered through a pipette containing flash silica gel, washing with Skellysolve B, then eluting with chloroform-ethyl acetate, 95:5 to yield a mixture (2:8.5:1) of starting material 119, monobromide 120, and dibromide 121. The physical properties of the mono- and dibromides were obtained from the above mixture. Compound 120 has the following physical properties. tlc: R_f 0.50 (chloroform-ethyl acetate, 90:10); hreims: no M^+; m/z calcd.

for $C_{12}H_9O_5{}^{81}Br(M+2-C_2H_2O)$: 313.9613; found 313.9609(4); calcd. for $C_{12}H_9O_5{}^{79}Br(M^+-C_2H_2O)$: 311.9633; found 311.9635(4), 275(2), 272(34), 270(35), 191(100); cims: m/z 374(M+2+18), 372(M+18); ^{1}H nmr($CDCl_3$): δ2.34(3H, s, $OCOCH_3$), 2.42(3H, s, $OCOCH_3$), 4.20(2H, s, CH_2), 6.56(1H, s, CH), 7.31(1H, d, J=8.5 Hz, ArH), 7.54(1H, d, J=8.5 Hz, ArH).

Compound 121 has the following physical properties. tlc: R_f 0.59 (chloroform-ethyl acetate, 90:10); hreims: no M^+; m/z calcd. for $C_{12}H_8O_5{}^{81}Br_2(M+4-C_2H_2O)$: 393.8698; found 393.8703(0.6); calcd. for $C_{12}H_8O_5{}^{81}Br{}^{79}Br(M+2-C_2H_2O)$: 391.8718; found 391.8713(1.2); calcd. for $C_{12}H_8O_5{}^{79}Br_2(M^+-C_2H_2O)$: 389.8738; found 389.8736(0.6), 352(6), 350(11), 348(6); cims: m/z 454(M+4+18), 452(M+2+18), 450(M+18); ^{1}H nmr($CDCl_3$): δ2.35(3H, s, $OCOCH_3$), 2.43(3H, s, $OCOCH_3$), 6.29(1H, s, $CHBr_2$), 6.75(1H, s, vinylic CH), 7.38(1H, d, J=8.5 Hz, ArH), 7.58(1H, d, J=8.5 Hz, ArH).

Attempted displacement of bromine in 120 using silver carbonate

A solution of monobromide 120 and dibromide 121 (ca. 2:1, 15 mg) in acetone (2 mL) was cooled to 0°C in an ice bath. Water (1 drop) was added, followed by silver carbonate (13 mg). The solution was magnetically stirred in the dark for 1 hour, then allowed to warm to room temperature and stirred for 22 hours. The reaction mixture was filtered and evaporated to dryness in vacuo. ^{1}H nmr analysis of the crude material indicated no change in the

mixture of mono- and dibromides.

3-acetoxy-methyl-7,8-diacetoxyisocoumarin 122

The mixture (2:8.5:1, vide supra) of diacetate 119, monobromide 120, and dibromide 121 was dissolved in dry benzene (25 mL). Silver acetate (0.131 g) was added and the magnetically stirred solution was refluxed in the dark for 12 days. Dry benzene was added periodically to maintain the volume of the reaction mixture. Additional silver acetate (0.131 g) was added and refluxing was continued for 5 days. Ater cooling to room temperature the reaction mixture was filtered and the solid residue washed with ethyl acetate. The filtrate was evaporated to dryness in vacuo. The crude material (27 mg) was subjected to flash chromatography (1 cm column, sample dissolved in chloroform, elution with Skellysolve B-ethyl acetate-acetic acid, 65:35:1) to yield diacetate 119 (4 mg), triacetate 122 (10 mg, 33% from diacetate 119), and aldehyde 123 (1 mg). Compound 122 has the following physical properties. mp: 136-138°C (ethyl acetate); tlc: R_f 0.29 (chloroform-ethyl acetate, 90:10); ftir($CHCl_3$ cast): 1776, 1741, 1491, 1371, 1204, 1186, 1157 cm^{-1}; hreims: m/z calcd. for $C_{16}H_{14}O_8(M^+)$: 334.0689; found 334.0686(7), 292(6), 250(100), 208(39), 207(22); ^1H nmr($CDCl_3$): δ2.15(3H, s, CH_2OCOCH_3), 2.35(3H, s, $OCOCH_3$), 2.42(3H, s, $OCOCH_3$), 4.87(2H, s, CH_2), 6.54(1H, s, CH), 7.33(1H, d, J=8.5 Hz, ArH), 7.55(1H, d, J=8.5 Hz, ArH).

Compound 123 has the following physical properties. tlc: R_f 0.19 (chloroform-ethyl acetate, 90:10); ftir($CHCl_3$ cast): 1776, 1743, 1713, 1371, 1199, 1185, 1156, 1016 cm^{-1}; hreims: m/z calcd. for $C_{14}H_{10}O_7(M^+)$: 290.0426; found 290.0425(4), 248(10), 206(100), 177(24); ^1H nmr($CDCl_3$): δ2.37(3H, s, $OCOCH_3$), 2.45(3H, s, $OCOCH_3$), 7.25(1H, s, vinylic CH), 7.57(1H, d, J=8.5 Hz, ArH), 7.68(1H, d, J=8.5 Hz, ArH), 9.59(1H, s, CHO).

3-hydroxymethyl-7,8-dihydroxyisocoumarin 15

Triacetate 122 (5.8 mg, 1.74×10^{-5} mol) was dissolved in methanol (2 mL). Potassium carbonate (7.2 mg, 5.21×10^{-5} mol) was added to the magnetically stirred solution, resulting in the formation of a yellow colour. The reaction mixture was stirred for 3 minutes at room temperature, then cooled to ca. 5°C for 15 minutes (no stirring). Stirring at room temperature was resumed for 20 minutes. Acidification with 5% (v/v) hydrochloric acid caused the deep yellow reaction mixture to become almost colourless. Brine (5 mL) was added and the products were isolated by extraction with ethyl acetate (3x 10 mL). The combined ethyl acetate extracts were dried over sodium sulphate, filtered, and evaporated to dryness in vacuo. The crude material (5 mg) was subjected to dry flash chromatography (0.5 cm column, Skellysolve B-ethyl acetate-acetic acid, 50:50:1) to yield trihydroxyisocoumarin 15 (0.7 mg, 19%). Compound 15 has the following physical properties. tlc: R_f 0.25 (Skellysolve

B-ethyl acetate-acetic acid, 50:50:1); ftir(CH_3COCH_3 cast): 3600-2600(b), 1686, 1290 cm^{-1}; hreims: m/z calcd. for $C_{10}H_8O_5(M^+)$: 208.0372; found 208.0371(100), 179(29), 177(19), 162(39), 149(42), 121(44); ^1H nmr(acetone-d_6): δ4.39(2H, s, CH_2), 4.66(1H, bs, CH_2OH), 6.65(1H, s, CH), 6.97(1H, d, J=8.25 Hz, ArH), 7.31(1H, d, J=8.25 Hz, ArH), 8.40(1H, bs, ArOH), 11.00(1H, bs, peri ArOH).

Attempted preparation of diketone 105 by selenium dioxide oxidation of ketone 77

To a solution of ketone 77 (34 mg, 1.16×10^{-4} mol) in acetic acid-water (7:3, 5 mL) was added selenium dioxide (14 mg, 1.28×10^{-4} mol). The magnetically stirred solution was heated at 85-88°C in a constant temperature oil bath for 14.5 hours. Saturated aqueous ammonium chloride (5 mL) was added and the reaction mixture was extracted with ether (3x 10 mL). The combined ether extracts were dried over sodium sulphate, filtered, and evaporated in vacuo. Examination of the crude material by tlc showed that 77 was still present. Thus the crude was resubjected to the above reaction conditions, using selenium dioxide (14 mg), and heating at 89°C for 17.5 hours. The crude products were isolated as described above. Flash chromatography (1 cm column, chloroform-ethyl acetate, 90:10) afforded aldehyde 99 (3.8 mg, 12%) as the only identifiable product.

The reaction was attempted in 95% ethanol (2 mL) using the following quantities: ketone 77: 39 mg, 1.33×10^{-4} mol;

selenium dioxide: 16 mg, 1.46×10^{-4} mol. The reaction was magnetically stirred at room temperature for 22.5 hours, then refluxed for 13 hours. The reaction mixture contained mostly starting material 77 and only trace amounts of products were apparent by tlc.

The reaction was also carried out in dry pyridine (7.5 mL) using the following quantities: ketone 77 (38 mg, 1.30×10^{-4} mol); selenium dioxide (32 mg, 2.86×10^{-4} mol. The magnetically stirred solution was refluxed for 19.5 hours. Upon cooling to room temperature water (5 mL) was added, and the reaction mixture was extracted with ethyl acetate (3x 10 mL). The combined ethyl acetate extracts were dried over sodium sulphate, filtered, and evaporated to dryness *in vacuo*. Examination of the crude products by tlc revealed a complex mixture which was not further investigated.

Attempted isomerisation of allylbenzamide 56 to olefin 147 using para-toluenesulphonic acid

To a solution of allylbenzamide 56 (1.024 g, 3.70×10^{-3} mol) in toluene (25 mL) was added *para*-toluenesulphonic acid monohydrate (0.070 g, 3.70×10^{-4} mol). The magnetically stirred solution was refluxed for 17.5 hours. Upon cooling to room temperature the reaction mixture was washed with aqueous sodium bicarbonate (10% w/v, 2 x 25 mL). The toluene extract was dried over sodium sulphate, filtered, and evaporated *in vacuo* to give unchanged starting material 56 (1.010 g). In resubjecting 56 to same conditions

described above, the following quantities were used: toluene: 25 mL; para-toluenesulphonic acid monohydrate: 0.696 g. After 5 days under reflux, additional para-toluenesulphonic acid (0.696 g) was added and refluxing was continued for 2 days. Upon cooling to room temperature an aqueous layer separated from the toluene layer. The toluene was washed with aqueous sodium bicarbonate (10% w/v, 25 mL). The aqueous layer was extracted with toluene (3x 25 mL). The combined toluene layers were washed with water (100 mL), dried over sodium sulphate, filtered, and evaporated in vacuo to give crude material (0.821 g). Examination of the crude reaction products by ^{1}H nmr indicated the presence of starting material 56, olefin 146, phthalide 88 (in a ratio of 2:1:1.5), lactone 75, and at least one unidentified component.

Olefin 147 via isomerisation of allylbenzamide 56 under basic conditions

Allylbenzamide 56 (0.996 g, 3.60×10^{-3} mol) was dissolved in methanolic sodium hydroxide (3 N, 25 mL) and the reaction mixture was refluxed for 68 hours. Upon cooling to room temperature water (25 mL) was added and the solution was neutralised with 6 N hydrochloric acid. The product was extracted with chloroform (3x 50 mL). The combined chloroform extracts were dried over sodium sulphate, filtered, and evaporated in vacuo. The crude product was filtered through a pipette containing flash

silica gel, eluting with ethyl acetate, to yield olefin 146 (0.990, 99%, 7:1 trans-cis). Distillation (137°C/0.07 Torr) provided an analytical sample. Compound 146 has the following physical properties. tlc: R_f 0.38 (Skellysolve B-ethyl acetate-acetic acid, 65:35:1); ftir($CHCl_3$ cast): 2937, 1635, 1487, 1289, 1061, 966 cm^{-1}; hreims: m/z calcd. for $C_{16}H_{23}NO_3(M^+)$: 277.1678; found 277.1689(31), 205(100); ^1H nmr($CDCl_3$): δ1.00(3H, t, J=7 Hz, $CH_2\underline{CH_3}$ of cis), 1.01(3H, t, J=7.5 Hz, $CH_2\underline{CH_3}$ of trans), 1.24(3H, t, J=7.25 Hz, $CH_2\underline{CH_3}$ of cis), 1.27(3H, t, J= 6.75 Hz, $CH_2\underline{CH_3}$ of trans), 1.82(3H, d, J=6.5 Hz, vinylic CH_3 of trans), 1.83(3H, d, J=6.5 Hz, vinylic CH_3 of cis), 3.09(4H, m, 2x $\underline{CH_2}CH_3$), 3.60(4H, m, 2x CH_2CH_3), 3.83(3H, s, OCH_3 of trans), 3.85(3H, s, OCH_3 of cis), 3.86(3H, s, OCH_3 of trans), 3.88(3H, s, OCH_3 of cis), 5.74(1H, dq, J=11, 6.5 Hz, CH=$\underline{CH}CH_3$ of cis), 6.10(1H, dq, J=15.75, 6.5 Hz, CH=$\underline{CH}CH_3$ of trans), 6.25(1H, bd, J=15.75 Hz, $\underline{CH}$=CH of trans), 6.29(1H, bd, J=11 Hz, $\underline{CH}$=CH of cis), 6.86(1H, d, J=8.5 Hz, ArH of trans), 6.88(1H, d, J=8.5 Hz, ArH of cis), 7.07(1H, d, J=8.5 Hz, ArH of cis), 7.19(1H, d, J=8.5 Hz, ArH of trans); ^{13}C nmr($CDCl_3$): δ12.3(2x), 13.4(2x), 14.2(cis), 18.2(trans), 55.5(2x), 61.1(2x)(CH_3); 38.4(trans), 42.4(cis), 42.5(trans), 42.9(cis)(CH_2); 111.4(cis), 112.3(trans), 121.0(2x), 125.0(cis), 126.0(trans), 126.8(cis), 126.9(trans)(CH); 127.7(2x), 130.5(trans), 132(cis), 144.4(2x), 151.1(2x)(C); 167.1(2x)(C=O); Analysis: calcd. for $C_{16}H_{23}NO_3$: C 69.29, H 8.36, N 5.05; found C 69.21, H 8.51, N 4.97.

N,N-diethyl-2,3-dimethoxy-6(1,2-pyruvyl)benzamide (105)

Olefin 146 (1.114 g, 4.02×10^{-3} mol) was dissolved in acetic anhydride (25 mL). The magnetically stirred solution was cooled to 4°C in an ice bath. Potassium permanganate (2.54 g, 1.61×10^{-2} mol, pulverised) was added in portions over 30 minutes, such that the temperature did not rise above 10°C. The reaction mixture was stirred for 3 hours at ca. 7°C. Ice cold ethyl acetate (50 mL) was added, followed by a solution of sodium bisulphite (3.35 g, 3.22×10^{-2} mol) in ice water (25 mL), resulting in an exothermic reaction. Stirring was continued for 5 minutes. Solid sodium chloride was added and the aqueous phase was extracted with ethyl acetate (3x 50 mL). The combined ethyl acetate layers were washed with 1 N aqueous sodium hydroxide (1x 200 mL) and water (1x 200 mL), dried over sodium sulphate, filtered, and evaporated in vacuo to give a deep yellow oil (1.095 g). Purification of the yellow oil by flash chromatography afforded keto-acetate 149 (0.052 g, 4%) and diketone 105 (0.504 g, 41%) by elution with chloroform-ethyl acetate, 75:25, and keto-acetate 150 (0.094 g, 7%) by elution with chloroform-ethyl acetate, 50:50.

The reaction was also carried out exactly according to the literature[98] procedure. The following quantities were used: olefin 146: 0.552 g, 1.99×10^{-3} mol; acetic anhydride: 10 mL; potassium permanganate: 1.26 g, 7.96×10^{-3} mol, pulverised; sodium bisulphite: 1.66 g, 1.59×10^{-2} mol. Potassium permanganate was added in portions over 15

minutes. The reaction mixture was magnetically stirred at *ca.* 7°C for 2 hours. Ice cold Skellysolve B-ethyl acetate, 1:1 (20 mL) were added, followed by sodium bisulphite in ice water (10 mL), resulting in an increase in temperature to 21°C. Stirring was continued for 5 minutes. Solid sodium chloride was added and the organic layer was washed with water (3x 20 mL) and 1 N aqueous sodium hydroxide (1x 20 mL). Concentration of the organic phase *in vacuo* gave a bright yellow oil which was dissolved in pyridine (10 mL). The magnetically stirred solution was cooled in an ice bath and water (10 mL) was added dropwise. Stirring was continued for 10 minutes at room temperature. Skellysolve B-ethyl acetate, 1:1 (20 mL) was added, followed by water (20 mL). The organic layer was washed with 1 N aqueous sodium hydroxide (1x 20 mL), hydrochloric acid (10% v/v, 1x 20 mL), and water (1x 20 mL), dried over sodium sulphate, filtered, and evaporated *in vacuo* to afford a deep yellow oil (0.119 g). Flash chromatography (2 cm column) of the yellow oil yielded diketone 105 (0.092 g, 15%) by elution with chloroform-ethyl acetate, 75:25, and keto-acetate 150 (0.007 g, 1%).

Compound 149 exists as a pair of rotational isomers (3.3:1) and has the following physical properties. tlc: R_f 0.54 (chloroform-ethyl acetate, 50:50); ftir($CHCl_3$ cast): 1748, 1732, 1631, 1286, 1232, 1081 cm^{-1}; hreims: m/z calcd. for $C_{18}H_{25}NO_6(M^+)$: 351.1682; found 351.1698(2), 309(19),

266(22), 193(100); ^{1}H nmr($CDCl_3$): δ1.02(3H, t, J=7.25 Hz, $CH_2\underline{CH_3}$), 1.17(3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 1.27(6H, t, J= 7 Hz, 2x $CH_2\underline{CH_3}$), 2.13(6H, s, 2x $OCOCH_3$), 2.15(3H, s, $COCH_3$), 2.18(3H, s, $COCH_3$), 3.02-3.33(4H, m, 2x CH_2), 3.41-3.78(4H, m, 2x CH_2), 3.84(3H, s, OCH_3), 3.87(3H, s, OCH_3), 3.91(6H, s, 2x OCH_3), 5.89(1H, s, CHOAc), 5.91(1H, s, CHOAc), 6.94(1H, d, J=8.5 Hz, ArH), 6.96(1H, d, J=8.5 Hz, ArH), 7.08(1H, d, J=8.5 Hz, ArH), 7.11(1H, d, J=8.5 Hz, ArH).

Diketone 105 has the following physical properties. bp 180°C/0.075 Torr; tlc: R_f 0.39(chloroform-ethyl acetate, 50:50); ftir(CH_2Cl_2 cast): 1713, 1669, 1636, 1615, 1589, 1569, 1282 cm^{-1}; uv(CH_3OH): 206(ε8830), 296(ε3060); hreims: no M^+; m/z calcd. for $C_{14}H_{18}NO_4$(M^+-$COCH_3$): 264.1236; found 264.1239(100), 236(26), 190(26); cims: m/z 308(M+1); ^{1}H nmr($CDCl_3$): δ1.10(3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 1.28(3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 2.46(3H, s, $COCH_3$), 3.18(2H, q, J=7 Hz, $\underline{CH_2}CH_3$), 3.52(1H, dq, J=14, 7 Hz) and 3.62(1H, dq, J=14, 7 Hz, $\underline{CH_2}CH_3$), 3.82(3H, s, OCH_3), 3.96(3H, s, OCH_3), 6.95(1H, d, J=9 Hz, ArH), 7.72(1H, d, J=9 Hz, ArH); ^{13}C nmr($CDCl_3$): δ12.3, 13.2, 26.0, 56.0, 61.5(CH_3); 38.6, 43.1(CH_2); 111.4, 129.6(CH); 123.1, 134.1, 145.2, 157.4, 166.2(C); 190.5, 199.9(C=O).

Compound 150 exists as a pair of rotational isomers (3:1) and has the following physical properties. tlc: R_f 0.24 (chloroform-ethyl acetate, 50:50); ftir(CH_2Cl_2 cast): 1742, 1696, 1634, 1570, 1477, 1460, 1412, 1283, 1254, 1241, 1073, 1054 cm^{-1}; hreims: m/z calcd. for $C_{18}H_{25}NO_6$(M^+):

351.1682; found 351.1679(1), 279(3), 264(100), 237(37), 220(26), 72(26); ^{1}H nmr($CDCl_3$): δ1.00(3H, t, J=7.25 Hz, $CH_2\underline{CH_3}$), 1.06(3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 1.27(3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 1.29(3H, t, J=7.25 Hz, $CH_2\underline{CH_3}$), 1.47(6H, d, J=7 Hz, 2x $CH(OAc)\underline{CH_3}$), 2.10(3H, s, $OCOCH_3$), 2.14(3H, s, $OCOCH_3$), 3.04(2H, q, J=7.25 Hz, $\underline{CH_2}CH_3$), 3.12(2H, q, J=7 Hz, $\underline{CH_2}CH_3$), 3.49(2H, m) and 3.66(2H, m, 2x $\underline{CH_2}CH_3$), 3.83(3H, s, OCH_3), 3.85(3H, s, OCH_3), 3.95(6H, s, 2x OCH 3), 5.84(1H, q, J=7 Hz, $\underline{CH}(OAc)CH_3$), 5.91(1H, q, J=7 Hz, $\underline{CH}(OAc)CH_3$), 6.91(1H, d, J=8.5 Hz, ArH), 6.92(1H, d, J=8.5 Hz, ArH), 7.62(1H, d, J=8.5 Hz, ArH), 7.67(1H, d, J=8.5 Hz, ArH).

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